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A CHEMICAL STUDY OF THE FLESH OF EMACIATED CATTLE [1]

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WORK OF OTHER INVESTIGATORS

Emaciation in cattle, in the absence of recognizable disease, may be regarded as due to lack of feed, to malnutrition, or to a combination of these factors. So far as this condition is caused by lack of feed, the work of Trowbridge, Moulton, and Haigh (*13*) [2] is of interest. In their experiments seven thrifty, fat, yearling steers, as nearly uniform as it was practicable to obtain them, were used. One was slaughtered as a check at the beginning of the test. The others were fed a standard ration throughout the experiment in such quantities that two of them remained at approximately constant weight, two gained, and the other two lost approximately one-half pound each a day. The "maintenance" animals were slaughtered at the end of 6 and 12 months, respectively, the "submaintenance" animals at the end of 6 and 11 months, respectively, one of the "supermaintenance" steers at the end of 12 months, and the other discarded after 16 months. Of the steers receiving the submaintenance ration, the one slaughtered at the end of 6 months was in very poor condition and the other, slaughtered at the end of 11 months, was so extremely emaciated that it could scarcely have survived another month. In Table I are shown the apparent percentage changes in weight of the various parts and organs of the experimental animals, as calculated from the days found in the report.

It seems desirable to call particular attention to the data from steers Nos. 591 and 592, which were fed the submaintenance rations. The most noticeable thing is the relatively large decrease in the weights of the liver, pancreas, spleen, and thymus of each steer. The carcass weight of each steer also decreased very considerably, particularly that of steer No. 592, which was fed 11 months. The blood, kidneys, and intestines of these steers also decreased materially in weight, but the heart lost only slightly. On the other hand, the lungs of the submaintenance steers gained slightly in weight, and the brain and spinal cord made rather large gains.

The effect of the submaintenance ration on the deposits of fat in the carcasses of the two steers was pronounced. Steer No. 591 lost more than two-thirds and steer No. 592 more than 95 per cent of its adipose tissue during the feeding periods of 6 and 11 months, respectively. The fat content of the skeleton of steer No. 591 increased materially during the feeding period, but steer No. 592 lost

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[2] Reference is made by number (italic) to "Literature cited," p. 1013.

more than 75 per cent of the fat present at the beginning of the test. The marrow of the hollow bones of steer No. 592, which was fed the submaintenance ration for 11 months, contained practically no fat.

TABLE I.—*Effect of fattening, maintenance, and submaintenance rations on development of steers* [a]

Part of organ	Maintenance steers		Submaintenance steers		Fat steer
	No. 597 (6 months)	No. 595 (12 months)	No. 591 (6 months)	No. 592 (11 months)	No. 593 (6 months)
	Per cent change	*Per cent change*	*Per cent change*	*Per cent change*	*Per cent change*
Empty weight	+0.19	−1.60	−18.67	−30.67	+11.40
Carcass	−1.45	−1.83	−21.83	−36.77	+13.39
Blood	−4.90	+.72	−17.47	−16.71	−2.80
Heart (muscle)	−11.99	+14.64	−6.56	−.59	+2.59
Brain	−3.99	+17.08	+19.83	+16.34	+1.88
Spinal cord	+24.14	+83.68	+36.31	+39.42	+18.27
Liver	−40.78	−29.29	−57.90	−52.75	−24.42
Spleen	−17.62	+19.09	−27.79	−41.86	+5.75
Kidneys	−17.91	+1.64	−23.17	−6.32	−.47
Pancreas	−22.82	+41.03	−43.80	−44.48	−4.13
Thymus	+12.25	−21.21	−40.69	−83.58	+35.04
Stomachs	+15.78	+33.11	+.52	−2.37	+45.49
Intestines	−14.37	+2.93	−21.20	−7.11	+7.20
Lungs and trachea	−6.67	+1.70	+9.05	+.68	+2.36

[a] This table has been prepared by recalculating the data presented in Table 21 of the report by Trowbridge, Moulton, and Haigh (*13, p. 64–66*) so as to show the percentage change in weight of the different parts and organs of the animals during the feeding periods. The control animal slaughtered at the beginning of the test was used as the standard of reference.

The data in Table I show very clearly the pronounced effects of undernutrition on the development of the different parts and organs of the steer. In view of the marked losses suffered by most of the organs and by the carcass as a whole, it is noteworthy that some organs maintained their weight or even increased it.

In another publication Moulton (*11*) has reported the results of a chemical study of the flesh, blood, and liver of the same lot of steers, and in summarizing his results states, in part:

> Inanition or partial starvation does not cause a watery muscular tissue. Fat is almost entirely resorbed, while glycogen apparently is not * * *. The phosphorus content of the flesh is reduced 10 to 15 per cent.
>
> The liver has a somewhat higher water content in the starved animal, accompanied by a high nitrogen content. * * * The glycogen content is not depleted.
>
> The blood has more water and less nitrogen in the fasted steer.
>
> The muscle fibers have become very much smaller, but still are functioning muscle fibers.

Feder (*2*) determined the composition of the flesh of four emaciated cattle, but since two of the animals were affected with recognized diseases the analyses of these carcasses will not be discussed. The composition of the flesh of the other two emaciated cattle is shown in Table II.

TABLE II.—*Composition of flesh of emaciated cattle*

[Results are expressed in terms of percentages of fresh material] [a]

Description of sample	Moisture	Fat	Ash	Nonfatty organic matter	Ratio of nonfatty organic matter to moisture
1. Muscle from shoulder of 7-year-old cow which had become exhausted during transit on railway. Badly emaciated. No pathological changes in organs	*Per cent* 78.95	*Per cent* 1.33	*Per cent* 1.14	*Per cent* 18.58	1:4.25
2. Composite sample of very watery flesh from an animal so badly emaciated that practically all fat had been used up. No pathological changes in organs. Meat sold on "Freibank"	80.78	0.35	0.98	17.89	1:4.52

[a] The data in this table were taken from the report by Feder (*2*).

The bone marrow of sample No. 2, which was gelatinous and translucent in character, had the following composition: Moisture 93.13, fat 0.16, and ash 1.5 per cent, as compared with 92 per cent fat and 3.4 per cent moisture in normal bone marrow.

Feder also studied the distribution of notrogenous compounds in the water extract from a number of samples of flesh. He found that the water extract from those samples having the lower "Feder" numbers (ratio nonfatty organic matter to moisture) contained a higher proportion of nitrogenous compounds that were not precipitated by trichloracetic acid than did samples of flesh having higher "Feder" numbers. Feder states that in certain cases the flesh of poorly nourished or diseased animals may show higher ratio numbers than 4.0.

TERMS USED IN INVESTIGATION

Before presenting the results of this investigation it seems necessary to define the terms "very thin" and "extremely emaciated" as they will be employed in this paper. "Very thin" cattle may be defined as cattle with a much shrunken musculature and with practically no fat deposits, but with no apparent abnormal condition of flesh, connective tissue, bone marrow, or organs. "Extremely emaciated" cattle, on the other hand, may be defined as cattle in which emaciation has proceeded to such a degree that the flesh, connective tissue, and bone marrow have become distinctly abnormal in appearance. In this paper these terms will be applied to those animals only that are free from evidence of recognized disease.

MATERIAL USED IN INVESTIGATION

The material used in this investigation consisted of (1) hind quarters from extremely emaciated cattle, (2) hind quarters from very thin cattle. The quarters were obtained from meat-packing establishments in Chicago, Ill., and Kansas City, Kans. As soon as it was thoroughly chilled the beef was wrapped in cheesecloth and burlap and forwarded in refrigerator cars to the Biochemic Division, Bureau of Animal Industry, Washington, D. C.

On receipt at the laboratory the quarters were placed in cold storage at a temperature approximating 36° F. Each quarter was weighed, and a record was made of its physical characteristics. As a rule, the femur was sawed in two, so that the marrow could be examined. The muscle tissue was carefully separated from bone and connective tissue, ground twice, and approximately 2 pounds was transferred to a glass jar for analysis. The chemical determinations were begun either the same day that the meat was prepared for analysis or the following morning.

DESCRIPTION OF HIND QUARTERS FROM EXTREMELY EMACIATED CATTLE

In all, 20 hind quarters from a like number of extremely emaciated cattle were examined. It is not worth while to describe each quarter in detail, since all had the same general characteristics, which were as follows:

(1) There was practically complete absence of visible fat, although a slight deposit was found between the muscle bundles in one or two quarters. The

connective tissue about the kidneys, where fat had previously been deposited, was slimy in character.

(2) The marrow in the femur was gelatinous, translucent, and usually semi-fluid in character.

(3) All quarters were extremely emaciated, the musculature being much shrunken, particularly on the loin.

(4) The muscle tissue was soft and flabby, and often rather sticky on the cut surface; as a rule, it was very watery. In some instances the muscles were dark in color, in other cases light, but the bright red characteristic of fat beef was always absent.

(5) As a rule the connective tissue between the muscle bundles was watery and slimy.

The weights of individual quarters ranged from 49 to 124 pounds, the average being 76 pounds. The time which elapsed between the slaughter of the cattle and the receipt of the quarters of beef at the laboratory ranged from 5 to 12 days, the average being 7½ days. The meat was under refrigeration practically all this time.

DESCRIPTION OF HIND QUARTERS FROM VERY THIN CATTLE

Twelve hind quarters from very thin carcasses, corresponding to a like number of cattle, were examined. The weights of the quarters ranged from 47 to 86 pounds, the average being 61 pounds. The time which elapsed between the slaughter of the cattle and the receipt of the beef at the laboratory ranged from 5 to 7 days. Brief descriptions of the several quarters of beef are given in the following:

Samples Nos. 766, 767, 768, 770, 771, and 772 were very similar in appearance, being very thin with a much shrunken and rather flabby musculature. Kidney fat was practically lacking, and fat between the muscle bundles was scant. The marrow in the femur was firm.

In sample No. 999 the musculature was much shrunken, particularly over the loin, and the cut surface of the muscle was lighter in color than normal for fat beef. The connective tissue between the muscle bundles was somewhat watery. There was no fat on the exterior of the round or loin, and only a small quantity about the kidneys and between the muscle bundles. The marrow in the femur was gelatinous in appearance, but of firm consistency.

Sample No. 1000 was similar in appearance to No. 999 except that the connective tissue between the muscle bundles was rather more watery and the marrow in the femur was gelatinous in appearance and soft.

Sample No. 1004 was a thin quarter of beef with only slight deposits of fat between the muscle bundles and with practically no kidney fat. The muscle tissue was firm and of fair color, and the connective tissue was normal in appearance. The marrow in the femur was firm and appeared to contain some fat.

In sample No. 1005 the muscle tissue was rather soft, watery, and light in color. The connective tissue between the muscle bundles was somewhat watery. There was no kidney fat. This quarter of beef was in appreciably poorer condition than No. 1004.

Sample No. 1008 was a thin quarter of beef with no fat deposits. The muscle tissue was rather soft and the connective tissue between the muscle bundles was somewhat watery in character. The bone marrow was reasonably firm.

Sample No. 1010, which was from the carcass of a bull, was of rather better quality than any of the other quarters of beef in this group.

The musculature was fairly well developed and of firm consistency, and the freshly cut surface of the meat was normal in appearance. There was no kidney fat and only a little intramuscular fat. The marrow in the femur was of firm consistency. The connective tissue between the muscle bundles was a trifle watery.

METHODS OF ANALYSIS

For the most part the methods used were those previously described by the authors (*6*). Purines were determined by the method of Krüger and Schittenhelm (*9*), creatinine by Folin's method (*3*), glycogen by Pflüger's method as described by Grube (*5*), sugar by Hoag and's method (*7*), and urea by the urease method (*8*). All determinations were made in duplicate, and the averages of closely agreeing results are reported.

COMPOSITION OF FLESH FROM EXTREMELY EMACIATED CATTLE

At first, in order to ascertain in what important respects, if any, the flesh of this class of cattle differs in composition from that of fat cattle, a rather complete analysis was made of the muscle tissue from each of three hind quarters obtained from a like number of extremely emaciated cattle. These data are reported in Table III, together with analyses of fat cattle obtained in a previous investigation. Several very marked differences in the composition of the lean meat from the two classes of cattle are apparent. The flesh from the extremely emaciated cattle is characterized by a much higher moisture content and by a wider ratio between protein and moisture than the flesh from the fat cattle. On the other hand, the flesh from fat cattle contains an appreciably higher proportion of each of the following constituents: Ash, ether extract, total nitrogen, total protein, total phosphorus, soluble phosphorus, soluble organic phosphorus, and free acid. The percentages of purine and creatinine in the flesh of the extremely emaciated cattle are within normal limits, but the apparent absence of sugar is noteworthy, since good-quality beef usually contains between 0.15 and 0.50 per cent dextrose.

TABLE III.—*Composition of flesh from extremely emaciated cattle as compared with that from fat cattle*

[Results are expressed in terms of percentages of the fresh material]

Constituent	Extremely emaciated cattle			Fat cattle, average of 5 steers
	No. 689	No. 707	No. 711	
	Per cent	*Per cent*	*Per cent*	*Per cent*
Moisture	79.96	79.75	81.39	74.20
Ash	.96	1.01	.96	1.07
Ether extract	.43	.34	.38	2.71
Total nitrogen	3.14	3.14	2.88	3.44
Protein (N×6.25)	19.63	19.63	18.00	21.50
Ratio, protein to moisture	1:4.1	1:4.1	1:4.5	1:3.5
Soluble nitrogen	.74	.98	.96	.98
Coagulable nitrogen	----	.59	.62	.55
Amino nitrogen	.109	.085	.073	.089
Purine nitrogen	.050	.055	.055	----
Purine	.110	.120	.120	----
Creatinine	.35	.37	.30	----
Dextrose	None.	None.	----	----
Total phosphorus	.175	.177	.158	.203
Soluble phosphorus	.136	.136	.125	.155
Soluble inorganic phosphorus	.117	.120	.110	.111
Soluble organic phosphorus	.019	.016	.015	.044
Acidity	.50	.42	.35	.74

In order to correct for the effects of the different percentages of fat present in the flesh of the two classes of cattle on the content of the other constituents the results of the analyses have been calculated to the fat-free basis, and these data are presented in Table IV. It is apparent that the composition of the fat-free flesh of the extremely emaciated cattle is not materially different from that of the original material, since the fat content of these samples was very low. In the case of the fat cattle, however, the percentage of each of the constituents, except fat, is materially raised when the results are calculated as percentages of the fat-free material, but the same general differences in the composition of the flesh of the two classes of cattle remain.

TABLE IV.—*Composition of flesh from extremely emaciated cattle compared with that from fat cattle*

[Results are expressed in terms of percentages of the fat-free material]

Constituent	Extremely emaciated cattle			Fat cattle, average of 5 steers
	No. 689	No. 707	No. 711	
	Per cent	*Per cent*	*Per cent*	*Per cent*
Moisture	80.31	80.05	81.70	76.27
Ash	.96	1.01	.96	1.10
Total nitrogen	3.15	3.15	2.89	3.54
Protein (N×6.25)	19.69	19.69	18.06	22.09
Soluble nitrogen	.74	.98	.96	1.01
Coagulable nitrogen	----------	.59	.62	.57
Amino nitrogen	.109	.085	.073	.091
Purine nitrogen	.050	.055	.055	----------
Purines	.110	.120	.120	----------
Creatinine	.35	.37	.30	----------
Total phosphorus	.176	.178	.159	.209
Soluble phosphorus	.137	.136	.125	.159
Soluble inorganic phosphorus	.118	.120	.110	.114
Soluble organic phorphorus	.019	.016	.015	.045
Acidity	.50	.42	.35	.76

The average moisture content of the three samples of flesh from the extremely emaciated cattle is 80.68 per cent as compared with 76.27 in that from the fat cattle, a difference of 4.41 per cent. The average percentages of ash in the flesh from the two classes of cattle are 0.98 and 1.10 per cent, respectively, and the average percentages of protein are 19.15 and 22.09 per cent. The percentage of total phosphorus is much higher in the flesh from the fat steers than in that from the emaciated cattle, the average percentages being 0.209 and 0.171, respectively. On the other hand, no material differences were found in the total soluble, coagulable, or amino nitrogen in the muscle tissue of the two classes of animals, which apparently indicates that extreme emaciation in cattle is not accompanied by an increase in the solubility of the muscle proteins.

It has been suggested that there might be an accumulation of certain end products of metabolism in the flesh of badly emaciated cattle resulting from the failure of the kidneys to function normally. It was found, however, that the percentages of purines and creatinine in the muscle tissue of this class of cattle were within normal limits.

When the data in Table IV were calculated to the moisture-free basis it was found that, except as to sugar, the flesh of the two classes of animals did not differ materially in composition. This indicates that the differences in composition reported in Table IV are due chiefly to the greater moisture content of the flesh from the emaciated cattle.

In continuing the chemical examination of emaciated carcasses the analytical work was restricted to those constituents which, in the light of the preliminary experiments, appeared to be of significance, viz, moisture, ash, fat, total nitrogen, and sometimes sugar and urea. Twenty hind quarters of beef from a like number of extremely emaciated cattle were examined and the results are reported in Table V.

TABLE V.—*Composition of flesh of extremely emaciated cattle*

[Results are expressed in terms of percentages of the fresh material]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Ether extract	Urea	Glycogen	Dextrose
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	*Per cent*
689	79.96	0.96	3.14	19.63	1:4.1	0.43	.....	.....	None.
707	79.75	1.01	3.14	19.63	1:4.1	.34	.....	.....	None.
711	81.39	.96	2.88	18.00	1:4.5	.38	.....	.....	.....
737	79.77	1.04	3.12	19.50	1:4.1	.93	.....	.....	.....
739	82.11	.88	2.77	17.31	1:4.7	.46	.....	.....	.....
747	79.68	.91	2.99	18.69	1:4.3	.71	.....	.....	.....
748	77.38	.96	3.24	20.25	1:3.8	1.13	.....	.....	.....
749	78.87	.99	3.26	20.38	1:3.9	.38	.....	.....	.....
752	79.59	1.07	3.11	19.44	1:4.1	.28	.....	.....	.....
809	80.97	.96	3.02	18.88	1:4.3	.29	.....	.....	.....
810	79.76	.98	3.12	19.50	1:4.1	.33	.....	.....	.....
811	81.24	1.05	2.91	18.19	1:4.5	.38	.....	.....	.....
900	81.20	.92	2.92	18.25	1:4.4	.62	.....	.....	.....
901	80.75	.96	2.90	18.13	1:4.5	.54	.....	.....	.....
1006	79.74	1.01	3.09	19.31	1:4.1	.45	0.012	None.	None.
1009	79.75	.94	3.02	18.88	1:4.2	.33	.012	None.	None.
1085	80.40	.99	2.96	18.50	1:4.3	.25	.022	.....	None.
1087	79.02	1.08	3.14	19.63	1:4.0	.23	.023	.....	None.
1100	80.21	1.00	2.85	17.81	1:4.5	.22	.006	.....	0.18
1101	80.31	1.03	2.89	18.06	1:4.4	.21	.....	.....	.16
Maximum	82.11	1.08	3.26	20.38	1:4.7	1.13	.....	.....	.....
Minimum	77.38	.88	2.77	17.31	1:3.8	.21	.....	.....	.....
Average	80.09	.99	3.02	18.90	1:4.2	.45	.....	.....	.....

The flesh of the extremely emaciated cattle was again found to be characterized by a high moisture content as compared with that of fat cattle. The percentages of moisture in the 20 samples of flesh reported in Table V range from 77.38 to 82.11, the average being 80.09. Eighteen samples contained in excess of 79 per cent of moisture. By referring to Table III it will be noted that the average moisture content of the flesh from five fat cattle is 74.20 per cent, or when the results are calculated on the fat-free basis the percentage of moisture is 76.27 (Table IV).

The percentage of ash in the muscle tissue from the emaciated cattle ranges from 0.88 to 1.08, the average being 0.99. These figures are to be compared with an average of 1.07 per cent ash in the muscle tissue from fat cattle (Table III) or to 1.10 per cent in the fat-free muscle tissue from the same animals (Table IV).

The protein content of the samples of flesh from the emaciated cattle ranges from 17.31 to 20.38 per cent, the average being 18.90 per cent. The percentage of protein in the flesh from fat cattle is 21.50 (Table III), or 22.09 in the fat-free tissue (Table IV).

The ratio between protein and moisture in the samples of flesh from the emaciated cattle ranges from 1:3.8 to 1:4.7, the average being 1:4.2. Seventeen of the twenty samples show ratios wid er

than 1:4.0. By referring to Table III it will be noted that the average ratio between protein and moisture in the flesh from fat cattle is 1:3.5.

The fat content of the flesh from the extremely emaciated cattle is very low, the percentages ranging from 0.21 to 1.13 per cent, the average being 0.45 per cent. Only one sample contains more than 1 per cent of fat. These results are to be compared with 2.71 per cent fat in the flesh from the fat cattle (Table III). It is to be remembered that these data represent analyses of the lean meat which had been trimmed as free from fat as practicable.

Urea was estimated in only five carcasses from extremely emaciated animals. The results obtained indicate considerable variation in the urea content of the flesh from this class of animal. If, on the basis of results reported by Hoagland and Mansfield (*8*), we take the normal urea content of healthy muscle to be from 0.015 to 0.018 per cent, it is evident from Table V that three of the carcasses contained distinctly subnormal amounts of urea, whereas two contained quantities somewhat above the normal. The low results suggest subnormal metabolic activity; the higher results a slight retention of urea. In general, however, the idea that even extreme emaciation in cattle is characterized by any appreciable retention of urea is not supported by these results.

Although it was to have been expected that the glycogen reserve of extremely emaciated animals would be small, the complete absence of glycogen and dextrose from the flesh of any living animal is scarcely in harmony with accepted views. Yet dextrose was found in but two of the eight samples of emaciated flesh examined for this constituent, and glycogen in neither of two samples. In view of the nature of these results it should be pointed out that they do not necessarily indicate that the flesh was free from carbohydrates at the time of slaughter, for post-mortem destruction of carbohydrates might have occurred. In the case of dextrose, also, one must reckon with the possibility that in the reduction with Fehling's solution the reduced copper oxide might have been held in solution by the substances that do not occur in appreciable quantities in normal meat. These possibilities, of course, require further investigation before any far-reaching conclusions as to the carbohydrate content of badly emaciated flesh can be drawn.

COMPOSITION OF FLESH FROM VERY THIN CATTLE

The quarters of beef that were used in these tests were of very poor quality, and the meat was suitable only for canning or for the manufacture of sausage.

The moisture content of the lean meat from the very thin cattle ranges from 76.81 to 80.49 per cent, the average being 78.84 per cent. Six of the twelve samples contain moisture in excess of 79 per cent. These figures are to be compared with an average moisture content of 80.09 per cent in the flesh from the extremely emaciated cattle, and 74.20 per cent in that from the fat cattle.

The percentage of ash in the flesh from the very thin cattle ranges from 0.93 to 1.10, the average being 1.03 per cent. The average ash content of the lean meat from the extremely emaciated cattle is 0.99 per cent, and that from the fat cattle 1.07 per cent.

The protein content of the lean meat from the very thin cattle ranges from 18.13 to 21.07 per cent, the average being 19.65 per cent. The average percentage of protein in the flesh from the fat cattle is 21.50, and that from the extremely emaciated cattle is 18.90.

The ratio of protein to moisture in the flesh of the very thin cattle ranges from 1:3.6 to 1:4.4, the average being 1:4.0. Four of the twelve samples show ratios wider than 1:4.0, and three samples show the latter ratio. The average ratio of protein to moisture in the lean meat from the extremely emaciated cattle is 1:4.2, and in the flesh from the fat cattle it is 1:3.5.

The fat content of the lean meat from the very thin cattle ranges from 0.36 to 2.02 per cent, the average being 0.75 per cent. Only two samples contain more than 1 per cent fat. These data are to be compared with an average fat content of 2.71 per cent in the lean meat from the fat cattle, and 0.45 per cent in that from the extremely emaciated cattle.

The average urea content of six samples of flesh from very thin cattle was 0.12 per cent, as compared with an average of 0.15 per cent in the lean meat from extremely emaciated carcasses.

Reducing sugar was determined in six samples of lean meat from very thin cattle, but only a trace of sugar was found in one sample.

TABLE VI.—*Composition of flesh of very thin cattle*

[Results are expressed in terms of percentages of the fresh material]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Ether extract	Urea	Dextrose
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
766	76.81	1.08	3.37	21.06	1:3.6	1.13	----------	----------
767	77.67	1.08	3.25	20.31	1:3.8	2.02	----------	----------
768	79.34	1.04	3.19	19.94	1:4.0	.39	----------	----------
770	79.96	1.01	2.99	18.69	1:4.3	.48	----------	----------
771	78.37	1.10	3.21	20.06	1:3.9	.48	----------	----------
772	78.52	1.07	3.23	20.19	1:3.9	.42	----------	----------
999	79.41	1.04	3.19	19.94	1:4.0	----------	0.013	Absent.
1000	80.49	.97	2.90	18.13	1:4.4	.76	.011	Absent.
1004	78.21	1.03	3.16	19.75	1:4.0	.88	.011	Absent.
1005	80.11	.96	2.95	18.44	1:4.3	.68	.013	Absent.
1008	79.69	.93	3.02	18.88	1:4.2	.59	.010	Absent.
1010	77.48	1.01	3.27	20.44	1:3.8	.36	----------	Trace.
Maximum	80.49	1.10	3.37	21.06	1:4.4	2.02	.013	----------
Minimum	76.81	.93	2.90	18.13	1:3.6	.36	.010	----------
Average	78.84	1.03	3.14	19.65	1:4.0	.75	.012	----------

In Table VII are reported the analyses of the samples of flesh from the very thin cattle, expressed in terms of percentages of the fat-free material. With the exception of two samples, Nos. 766 and 767, it will be noted that the composition of the fat-free flesh does not differ materially from that of the fresh material as reported in Table VI.

TABLE VII.—*Composition of flesh of very thin cattle*

[Results are expressed in terms of percentages of fat-free flesh.]

Sample No.	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture	Urea	Dextrose
	Per cent	*Per cent*	*Per cent*	*Per cent*		*Per cent*	*Per cent*
766	77.69	1.09	3.41	21.31	1:3.6	.....	.....
767	79.27	1.10	3.32	20.75	1:3.8	.....	.....
768	79.65	1.04	3.20	20.00	1:4.0	.....	.....
770	80.34	1.01	3.04	19.00	1:4.3	.....	.....
771	78.75	1.11	3.23	20.19	1:3.9	.....	.....
772	78.85	1.07	3.24	20.25	1:3.9	.....	.....
999 [a]	.....	.....	.....	.....	1:4.0	.....	.....
1000	81.10	.98	2.92	18.25	1:4.4	0.011	None.
1004	78.90	1.04	3.19	19.94	1:4.0	.011	None.
1005	80.66	.97	2.97	18.56	1:4.3	.013	None.
1008	80.16	.94	3.04	19.00	1:4.2	.010	None.
1010	77.76	1.01	3.28	20.50	1:3.8	.....	Trace.
Maximum	81.10	1.11	3.41	21.31	1:4.4	.013	.....
Minimum	77.69	.94	2.92	18.25	1:3.6	.010	.....
Average	79.38	1.03	3.17	19.80	1:4.0	.011	.....

[a] Results not calculated to fat-free basis on account of loss of fat determination.

The average composition of the fat-free flesh from each of the three classes of cattle—namely, fat, very thin, and extremely emaciated—are reported in Table VIII.

TABLE VIII.—*Average composition of flesh from extremely emaciated, from very thin, and from fat cattle*

[Results are expressed in terms of percentages of fat-free material]

Class of cattle	Moisture	Ash	Total nitrogen	Protein (N×6.25)	Ratio protein to moisture
	Per cent	*Per cent*	*Per cent*	*Per cent*	
Extremely emaciated	80.45	0.99	3.03	18.94	1:4.2
Very thin	79.38	1.03	3.17	19.80	1:4.0
Fat	76.27	1.10	3.54	22.09	1:3.5

NOTE.—Data in question were calculated from Table V, which includes all extremely emaciated carcasses examined, rather than from Table IV, which included only the first three.

DISCUSSION OF RESULTS

Emaciation in cattle, except that caused by recognizable disease, must be considered as due to inadequate or faulty nutrition. It is a condition which develops when the destruction of body tissues takes place more rapidly than the processes of repair and growth, the nutritive reserves of the body becoming exhausted and the animal being obliged to utilize the muscle and other tissues in order to support life. Emaciation naturally differs in degree according to the extent to which the animal has used up its own tissues in order to meet its nutritive requirements. From the standpoint of food control it is important to know when this process of disintegration has proceeded to such an extent as to render the flesh of an animal unsuited for human consumption.

The investigation which was conducted by Trowbridge, Moulton, and Haigh (*13*) has furnished valuable information concerning emaciation in cattle caused by lack of feed. Particularly interest-

ing are the effects of long-continued undernutrition on the weights of the carcasses and organs of steers, as shown in Table I. In addition to these data it is reported that the steer that had been fed the submaintenance ration for the shorter period (6 months) had lost more than two-thirds of its adipose tissue, and the animal fed the same ration for the longer period (11 months) had lost 95 per cent of its fatty tissue. Also, the bone marrow of the latter steer contained practically no fat.

Moulton (*11*) determined the composition of the flesh of the above-mentioned steers, and as a result he concludes that "inanition or partial starvation does not cause a watery musculature." Although this statement is fully warranted as applying to the two animals in question, it is hardly justified as applying to emaciated cattle in general, as is apparent from the analyses which have been made of the flesh of cattle in advanced stages of emaciation (Tables III and IV), and from the work of Feder (*2*) (Table II).

From an examination of the data which have been presented herein concerning the composition of the flesh of fat, extremely emaciated, and very thin cattle, it is apparent that the flesh of the extremely emaciated cattle differs very materially from that of the fat cattle, and to a lesser extent from that of the very thin cattle. Although these differences are apparent in the moisture and in the protein content of the flesh from the two classes of animals, they are manifested more clearly and consistently in the protein-moisture ratio.

This ratio has already found use in the detection of water added to sausage, in which connection a ratio of 1: 4.0 is generally recognized as the upper limit for moisture in fresh, sound meat. Thus, as a consequence of investigations carried on from 1909 to 1912 by E. A. Boyer, in charge of the meat-inspection laboratory at Omaha, Nebr., this ratio has been used in the laboratories of the Bureau of Animal Industry since 1913 as a basis for the detection of added water in sausage. Although the result of Boyer's investigation has never been published, reference is made to it in the report of the Chief of the Bureau of Animal Industry for the fiscal year ended June 30, 1913 (*10*), in which it is stated that the addition of water to sausage may be detected by determining the protein-moisture ratio of the finished product.

The use of the ratio of protein to moisture, or of nonfatty organic matter to moisture, has also been used for some time in Germany as a basis for detecting added water in chopped meat or sausage. Thus Feder (*1*), in one of his earlier communications, recommended the use of the ratio between the fat-free organic matter and water as a basis for the detection of added water in meat-food products, pointing out that this ratio is fairly constant for fresh meats, and in case of beef or pork sausage meats does not vary appreciably from 1: 4.0.

This early work of Feder has been confirmed by later work by the same author and by that of other German investigators, so that now the ratio of nonfatty organic matter to moisture appears to have found widespread use in Germany as a means of detecting added water in sausage and minced meat. The ratio of 1: 4.0 is generally accepted as the standard for normal meat.

Grossfeld (*4*) found that the protein content ($N \times 6.25$) did not differ appreciably from that of organic nonfatty material, and so recommended the use of the ratio between protein and moisture

(1: 4.0) as a standard for the detection of added water, remarking that the value for protein is more quickly, more simply, and more cheaply obtained than that for fat-free organic matter.

Pannwitz and Harder (*12*) report the analyses of 102 samples of ground fresh beef, of which only 2 show protein-moisture ratios wider than 1:4.0, and it was found that these contained added water and salt.

Referring to the writers' investigations, it will be noted from Table V that the ratio of protein to moisture in the 20 samples of flesh from extremely emaciated cattle ranges from 1: 3.8 to 1: 4.7, the average being 1: 4.2. Seventeen of the twenty samples show ratios wider than 1: 4.0. It may be noted also that the three samples having the narrowest ratios likewise contain the lowest percentages of moisture, and two of the three samples contain the highest percentages of protein.

From Table VII, in which is shown the composition of the flesh from 12 very thin cattle, it will be noted that the ratio of protein to moisture ranges from 1: 3.6 to 1: 4.4, the average being 1: 4.0, the maximum standard set by Feder for normal flesh. Four of the twelve samples of flesh, Nos. 770, 1000, 1005, and 1008, show ratios wider than 1: 4.0, namely, 1: 4.3, 1: 4.4, 1: 4.3, and 1: 4.2, respectively. It may be noted also that these four samples contain higher percentages of moisture (fat-free basis) than any of the other samples from this group of cattle, each containing in excess of 80 per cent, and that they also contain lower percentages of protein than the other samples.

In describing the several quarters of beef from very thin cattle attention has already been called to the watery condition of the intramuscular connective tissue of samples Nos. 1000, 1005, and 1008. The flesh from each of these quarters of beef had a protein-moisture ratio wider than 1: 4.0. Although sample No. 770 had a ratio of 1: 4.3, the description of this quarter of beef does not indicate that the flesh or connective tissue appeared to be more watery than that of other quarters of beef having ratios of 1: 4.0 or narrower.

As judged by its percentages of protein and moisture and by the ration of protein to moisture, it thus appears that the flesh from four carcasses which had been classed as very thin resembled very closely that of other carcasses that had been classed as extremely emaciated. Whether this similarity indicates an error in the original classification of the carcasses on the basis of physical characteristics, or whether it indicates that the protein-moisture ratio is not an infallible index of the condition of the carcass, is not entirely clear. However, in view of the difficulty experienced in making a rational classification on the basis of physical appearance alone, the writers are inclined to the former alternative. But whichever alternative we accept, it is still evident that in the great majority of cases the protein-moisture ratio can be satisfactorily correlated with the physical condition of the carcass, and it is believed that this ratio may serve a useful purpose in distinguishing mere thinness from extreme emaciation in cattle.

CONCLUSIONS

The results of the investigation reported in this paper appear to justify the following conclusions:

1. The flesh of extremely emaciated cattle is characterized by a relatively high moisture content and by a low content of fat, protein, ash, and probably of sugar.

2. The ratio of protein to moisture in the flesh of extremely emaciated cattle, with but rare exceptions, is wider than 1:4.0, whereas the ratio for the flesh of normal cattle is usually much narrower.

3. It is believed that the ratio between protein and moisture in the flesh of "very thin" or "extremely emaciated" cattle will prove to be of value in classifying such animals for food purposes.

LITERATURE CITED

(1) FEDER, E.
1913. EINE GRUNDLAGE ZUR ERKENNUNG EINES ÜBERMÄSSIGEN WASSERZUSATZES ZU ZERKLEINERTEN FLEISCHWAREN. Ztschr. Untersuch. Nahr. u. Genussmtl. 25: 577–588.

(2) ———
1922. ZUR ZUSAMMENSETZUNG VON FLEISCHWAREN, BESONDERS VON WASSERREICHEM FLEISCH. Ztschr. Untersuch. Nahr. u. Genussmtl. 43: 193–199.

(3) FOLIN, O.
1914. ON THE DETERMINATION OF CREATININE AND CREATINE IN BLOOD, MILK AND TISSUES. Jour. Biol. Chem. 17: 475–481.

(4) GROSSFELD, J.
1921. DIE ERMITTELUNG DES WASSERZUSATZES IN HACKFLEISCH UND FLEISCHWÜRSTEN. Ztschr. Untersuch. Nahr. u. Genussmtl. 42: 173–181.

(5) GRUBE, K.
1910. NACHWEIS, DARSTELLUNG UND QUANTITATIVE BESTIMMUNG DES GLYKOGENS. *In* Abderhalden, E., Handbuch der Biochemischen Arbeitsmethoden, Bd. 2, p. 159–166. Berlin and Wien.

(6) HOAGLAND, R., MCBRYDE, C. N., and POWICK, W. C.
1917. CHANGES IN FRESH BEEF DURING COLD STORAGE ABOVE FREEZING. U. S. Dept. Agr. Bul. 433, 100 p., illus.

(7) ———
1917. THE QUANTITATIVE ESTIMATION OF DEXTROSE IN MUSCULAR TISSUE. Jour. Biol. Chem. 31: 67–77.

(8) ——— and MANSFIELD, C. M.
1917. THE FUNCTION OF MUSCULAR TISSUE IN UREA FORMATION. Jour. Biol. Chem. 31: 487–499.

(9) KRÜGER, M., and SCHITTENHELM, A.
1905. DIE MENGE UND HERKUNFT DER PURINKÖRPER IN DEN MENSCHLICHEN FAECES. II. MITTEILUNG. Ztschr. Physiol. Chem. 45: 14–27.

(10) MELVIN, A. D.
1913. REPORT OF THE CHIEF OF THE BUREAU OF ANIMAL INDUSTRY. U. S. Dept. Agr., Bur. Anim. Indus. Ann. Rpt. 1913, 34 p.

(11) MOULTON, C. R.
1920. BIOCHEMICAL CHANGES IN THE FLESH OF BEEF ANIMALS DURING UNDERFEEDING. Jour. Biol. Chem. 43: 67–78. illus.

(12) PANNWITZ and HARDER.
1922. BEITRÄGE ZUR KENNTNIS DER FEDER'SCHEN ZAHL. Ztschr. Untersuch. Nahr. u. Genussmtl. 44: 344–348.

(13) TROWBRIDGE, P. F., MOULTON, C. R., and HAIGH, L. D.
1918. EFFECT OF LIMITED FOOD SUPPLY ON THE GROWTH OF YOUNG BEEF ANIMALS. Mo. Agr. Exp. Sta. Research Bul. 28, 129 p., illus.

COMPOSITION OF MARROW OF FRESH AND CURED HAMS[1]

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During a recent conference of meat inspectors of the Bureau of Animal Industry engaged in the supervision of operations of establishments at which Federal meat inspection is maintained, the subject of marrow souring in ham bones was discussed. The question arose whether curing action takes place in the marrow of these bones. Discussion developed the fact that it was not definitely known whether curing took place in the marrow of the bones, and there were sharp differences of opinion among the inspectors on this point. The writers have since examined the marrow of the femur bones from a number of fresh and cured hams in order to determine whether chemical changes had taken place during the curing process.

The femur bones were cut out intact from the hams, all adhering particles of meat were carefully removed, and the bones were washed, in order that the pickle would not come in contact with the marrow. The bones were then cracked, the marrow was removed and the marrow from several hams was combined for each sample, care being taken that no particles of bone were included. The marrow was thoroughly mixed, and the sample was then analyzed.

The methods of the Association of Official Agricultural Chemists were followed in making all determinations. Results of the analyses were as follows:

Marrow from Femur Bones of Fresh Hams

Water	Protein	Fat	Ash	Sodium chloride	Nitrites
8.48	1.6	90.0	0.37	0.14	None.
10.90	1.5	87.7	.19	.14	None.
8.95	1.3	89.9	.17	.12	None.

Marrow from Femur Bones of Hams Cured by Usual Process

13.76	2.5	82.1	1.03	0.60	15 parts per million (as $NaNO_2$).
10.58	2.8	85.8	.95	.41	20 parts per million (as $NaNO_2$).
9.52	2.3	87.8	.60	.47	55 parts per million (as $NaNO_2$).

Marrow from Femur Bones of Hams Cured by Special Process

8.72	1.6	90.0	0.71	0.35	15 parts per million (as $NaNO_2$).

[1] Received for publication July 27, 1925; issued January, 1926.

The hams cured by special process were cured in pickle in which sodium nitrite had been used as the color fixative instead of the sodium or potassium nitrate commonly used. The percentage of ash, sodium chloride, and nitrites found were higher in the cured samples than in the fresh. This shows that the curing agents had actually penetrated the marrow of the femur bone.

INACTIVATION OF VITAMIN A BY RANCID FAT[1]

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INTRODUCTION

There is, perhaps, little need to be concerned with the characteristics of rancid fat as a human food, since by reason of its impalatability and its unsoundness it is already under the ban of the consumer, producer, and food inspector alike. Nevertheless it has seemed desirable, in the course of studies on rancidity, to carry on a few feeding experiments with rancid fats, partly in order to satisfy a somewhat prevalent curiosity concerning their wholesomeness and partly because of the possible bearing of the results on animal nutrition.

No attempt has been made to evaluate the effect of slightly rancid fats fed in small quantities, as this would probably have entailed a longer study than the importance of the subject seemed to warrant. The chief concern has been rather to discover whether the development of rancidity tends to impair the food value of a fat, and information along this line has been sought by the shortest route possible, viz, by the feeding of intensely rancid fats in comparatively large quantities.

GENERAL PROCEDURE

Three experiments, in which sweet and rancid lard were fed to white rats, were conducted. In each experiment a number of rats selected for uniformity as to age, weight, and general health were divided into two lots, in such manner that each rat in the first lot should have, as far as possible, its counterpart as to sex and parentage in the second lot. One group, used as controls, was fed a ration which was adequate to all intents and purposes and which contained from 16 to 25 per cent of fresh lard, while the other group was given a similar ration containing a corresponding amount of intensely rancid lard. Each rat was confined in a separate cage, which was cleaned at frequent intervals. About twice a week the weight of each rat was charted. In case of the rats receiving the fresh lard, the rations were renewed before any noticeable rancidity had developed in the old ration; and in all cases both the new and the residual rations were weighed when the rations were renewed.

The two lots of fat employed in these experiments were good grades of kettle-rendered lard that had been prepared under observation at a local rendering plant and transported directly to the laboratory in completely filled glass-stoppered bottles. In each case part of the lard, in its original containers, was stored in the dark at 34° F., while the remainder was transferred to a large flask placed before a light window, where for a period of several weeks it was melted and

[1] Received for publication April 30, 1925, issued January, 1926.

aerated daily, care being taken to avoid a temperature greatly in excess of its melting point. When the lard was sufficiently rancid, as indicated by the Kreis test and its odor and taste, the feeding experiment was begun.

EXPERIMENT 1

The rations employed in experiment 1 were made up as follows, the components being intimately mixed in a mortar:

	Per cent
Dry commercial casein	14
Cassava starch	53
Dried baker's yeast	10
Dried egg yolk	3
Salt mixture (Drummond and Watson's formula [2])	4
Lard (sweet or rancid)	16
	100

The sweet fat employed gave a negative Kreis test, and a negative test for peroxides, and contained 0.18 per cent of free fatty acids calculated as oleic acid. The rancid fat gave a positive Kreis test when diluted with 25 parts of pure mineral oil, and an intense test for peroxides, and contained 2.76 per cent of free fatty acids calculated as oleic acid.

Twelve rats were used in this experiment, six of which received the sweet fat and six the rancid. Their ages and weights at the beginning of the experiment, together with their respective sexes and litter numbers, are indicated in Table I.

TABLE I.—*Description of rats used in experiment 1*

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			Days	*Grams*					*Days*	*Grams*	
1	Rancid	Female	32	45	69–C	7	Sweet	Male	32	41	69–C
2	do	Male	32	46	69–C	8	do	Female	33	39	69–D
3	do	Female	32	48	69–C	9	do	Male	33	40	69–D
4	do	do	32	48	69–C	10	do	do	34	40	69–B
5	do	Male	30	52	70–B	11	do	Female	30	50	70–B
6	do	do	30	49	70–D	12	do	do	30	40	70–D

During the earlier stages of the experiment the rats receiving the rancid lard seemed to suffer from a mild diarrhea, indicative of intestinal irritation, but this symptom gradually subsided as the experiment progressed. In general, also, as is shown by the growth curves in Figure 1 and by the record of total food consumption in Table II, the rats of the rancid series consumed less ration and gained less weight than the corresponding controls. Finally, on the eighty-eighth day of the experiment rat No. 3 developed ophthalmia, and on the same day the experiment was terminated.

At the end of the experiment a blood count was made on rat No. 5, which was in the poorest condition of all the rats receiving the rancid lard, and on rat No. 10, which received the sweet lard. No essential difference was noted in the number of red cells in the blood

[2] DRUMMOND, J. C., and WATSON, A. F. THE TESTING OF FOODSTUFFS FOR VITAMINS. Analyst 47: 237. 1922.

of the two animals, the count being 8,825,000 per cubic millimeter for rat No. 5 and 8,825,000 to 9,025,000 per cubic millimeter for rat No. 10.

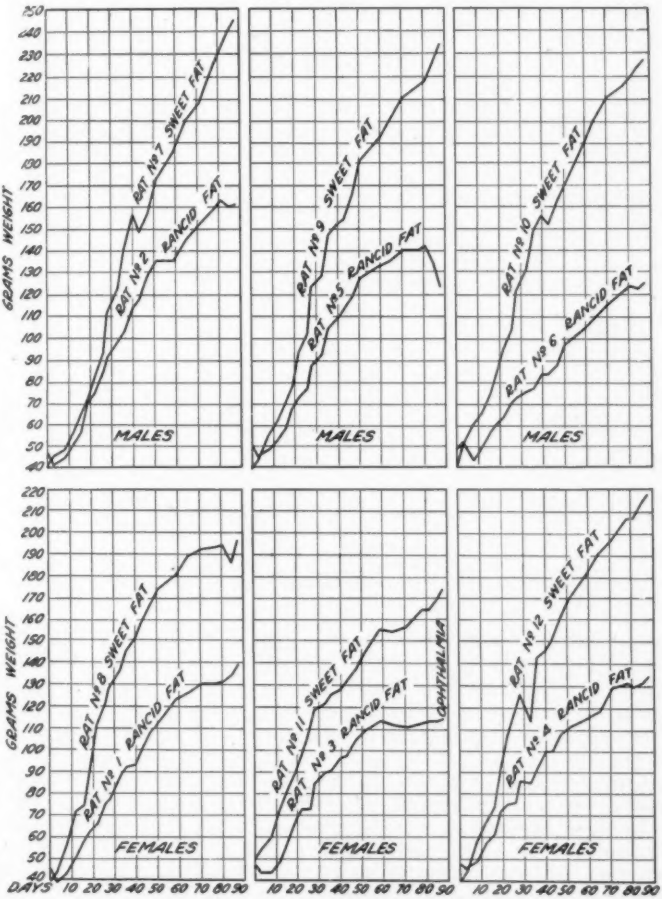

FIG. 1.—Growth curves of rats in experiment 1

Post-mortem examinations of rats Nos. 5 and 6 of the rancid series and of rats Nos. 7 and 10 of the control group yielded the following results:

Rat No. 6.—Practically no mesenteric or kidney fat. No noticeable congestion of mucous membrane of stomach or intestines. Slight congestion of mesenteric blood vessels. Mucous membrane of alimentary tract pale and unhealthy in appearance as compared with that of the controls. The same was true as to tissues in general. The general picture was that of undernutrition.

Rat No. 5.—Similar in condition to rat No. 6, except that there was even less visible fat and that the tissues appeared to be in a poorer state of nutrition. No congestion apparent.

Rat No. 7.—Well nourished, with plenty of fat. Viscera and muscles larger, firmer, and apparently more healthy than those of rats Nos. 5 and 6.

Rat No. 10.—Similar in condition to rat No. 7.

TABLE II.—*Food-consumption data for experiment 1, covering first 73 days*

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption	Total gain
			Grams	*Grams*	*Grams*
Rancid	2	Male	592	0. 0886	108
Do	5	do	538	.0797	89
Do	6	do	476	.0846	69
Average for males			535	.0843	89
Rancid	1	Female	508	.0827	85
Do	3	do	505	.0832	64
Do	4	do	539	.0857	82
Average for females			517	.0839	77
Average for males and females			526	.0841	83
Sweet	7	Male	709	.0854	171
Do	9	do	679	.0802	172
Do	10	do	699	.0835	172
Average for males			696	.0830	172
Sweet	8	Female	657	.0777	153
Do	11	do	594	.0768	109
Do	12	do	677	.0799	160
Average for females			643	.0781	141
Average for males and females			669	.0806	156

By interpolation of the growth curves and the records of food consumption, the weight of food consumed per day, per gram of weight of the given rat, was calculated for daily intervals. The average of the values thus obtained throughout the period covered by this table has been called the "coefficient of food consumption" for the rat in question.

In the light of these observations it would appear that rancid lard is not toxic to white rats, and that the subnormal growth of rats Nos. 1 to 6 must be attributed to undernutrition or to vitamin deficiency. Since the rancid ration may have been unpalatable, one immediately suspects that either or both of these conditions might have originated in a self-imposed curtailment of food consumption, and hence of vitamin-A intake, by the rats of the rancid series, under which circumstances even the development of ophthalmia in rat No. 3 would signify nothing as to the adequacy of the ration. But this suspicion is not supported by the food-consumption data as presented in Table II, for the rats of the rancid series consumed an even larger average weight of ration per day and per gram of body weight than the control rats receiving the sweet lard. Apparently, therefore, gross food consumption was limited by growth, not growth by the rate of food consumption; and the cause of this limitation of growth must be sought in the inferior quality of the ration containing the rancid fat as compared with the control ration.

Although the experiment may not be conclusive as to the nature of this inferiority, the development of ophthalmia in rat No. 3 suggests that it consisted in a deficiency of vitamin A, while the similarity of the two rations in other respects would indicate that the inferiority was caused by the presence of the rancid lard. In view of the known susceptibility of vitamin A to oxidation, and of the presence of organic

peroxides in rancid fat, and of the intimate admixture of the rancid lard with the ration fed to rats Nos. 1 to 6, one is inclined to suspect that the rancid lard effected a partial destruction of the vitamin A that was originally added to this ration.

EXPERIMENT 2

In experiment 2 vitamin A was fed separately from the main ration containing the lard, and was thus protected from any injurious contact with rancid fat. The supplemental ration containing the vitamin A was composed of 36 per cent of dried egg yolk and 64 per cent of starch, and was fed to all rats equally in daily portions ranging from 0.3 gram at the beginning of the experiment to 0.8 gram at the end. In this manner sufficient vitamin A was provided to cover the requirements of the most thrifty of the experimental animals.

The main portion of the ration, fed ad libitum, was made up as follows:

	Per cent
Dry commercial casein	13. 66
Cassava starch	47. 45
Dried baker's yeast	9. 76
Salt mixture [3]	3. 90
Lard (sweet or rancid)	25. 23
	100. 00

Naturally, each rat consumed a different amount of this ration, so that the quantitative composition of the total food derived from both rations varied from day to day and from rat to rat. The relative amount of the several food materials consumed by each group of rats as a whole, for the entire period of the experiment, however, was as follows:

	Per cent		Per cent
Sweet lard	22. 13	Rancid lard	20. 35
Casein	12. 00	Casein	11. 00
Starch	49. 47	Starch	50. 65
Dried yeast	8. 56	Dried yeast	7. 87
Salt mixture	3. 42	Salt mixture	3. 15
Dried egg yolk	4. 42	Dried egg yolk	6. 98
	100. 00		100. 00

The lard used in this experiment, though from a different lot, was of the same type and quality and was obtained and prepared in the same manner as that used in experiment 1. At the beginning of the experiment the sweet lard contained 0.32 per cent of free fatty acids calculated as oleic acid, and gave a negative Kreis test and a negative test for peroxides. The rancid lard, on the other hand, contained 0.48 per cent of free fatty acids calculated as oleic acid, and gave a positive Kreis test when diluted with 17 parts of pure mineral oil, and an intense test for peroxide.

Eight rats were used in this experiment, the pertinent data concerning which are recorded in Table III.

[3] DRUMMOND, J. C., and WATSON, A. F. Op. cit.

TABLE III.—*Description of rats used in experiment 2*

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			Days	*Grams*					*Days*	*Grams*	
13	Sweet	Male	25	48	112–B	17	Rancid	Male	25	46	112–B
14	do	Female	25	38	112–B	18	do	Female	25	49	112–B
15	do	Male	25	38	113–A	19	do	Male	25	37	113–A
16	do	Female	25	39	113–A	20	do	Female	[a] 26	37	113–A

[a] Rat No. 20 was started one day later than the others.

Contrasted with the conspicuous greed of the rancid group for the vitamin-A supplement was the comparative indifference toward it of rat No. 14 and in smaller degree of rats Nos. 13 and 16 of the control group. All the rats in the rancid group, as well as rat No. 15 in the

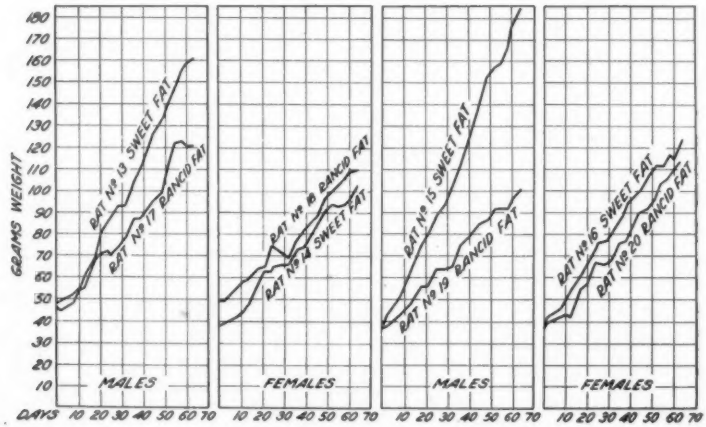

FIG. 2.—Growth curves of rats in experiment 2

control group, regularly consumed their daily supplements within a five-minute period, while rat No. 14 scarcely touched the supplemental ration during the first week of the experiment, and consumed it with but little interest and for the most part incompletely during the remainder of the experiment. Rats Nos. 13 and 16, on the other hand, while they ate the supplemental ration incompletely at the beginning of the experiment, consumed it completely and with an increasing show of interest in the later stages of the experiment. Throughout the experiment all the rats remained healthy and active and free from ophthalmia or other evidence of deficiency disease.

The growth curves for this experiment are presented in Figure 2, and the food-consumption data in Table IV. Evidently no appreciable limitation in the growth of the female rats in the rancid series was occasioned by the fact that they consumed slightly less ration per day per gram of body weight than the females in the control series. Presumably, therefore, diminished food consumption was not the limiting factor in the growth of the male rats of the

rancid series, since the rate of food consumption of the two series was more nearly equal in the case of the males than in the case of the females.

TABLE IV.—*Food-consumption data for experiment 2, covering first 60 days*

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption [a]	Total gain
			Grams	*Gram*	*Grams*
Sweet	13	Male	406	0.0705	110
Do	15	do	342	.0558	138
Average for males			374	.0631	124
Sweet	14	Female	280	.0691	58
Do	16	do	274	.0573	76
Average for females			277	.0632	67
Average for males and females			325	.0632	95
Rancid	17	Male	300	.0631	75
Do	19	do	232	.0578	59
Average for males			266	.0604	67
Rancid	18	Female	290	.0665	60
Do	20	do	209	.0508	72
Average for females			249	.0586	66
Average for males and females			258	.0595	66

[a] See footnote to Table II.

Yet it is somewhat remarkable that, whereas an approximate equivalence in rate of growth should have obtained between the two groups of females, the males of the control series should have grown so much more rapidly than the corresponding males receiving the rancid lard. In view of the fact that the latter rats received fully as much vitamin A as the former, it is difficult to understand the reason for their subnormal growth.

One might, of course, postulate that animal appetite is largely determined by nutritive requirements, and that the greater greed with which the rats of the rancid series consumed their vitamin-A supplements was conditioned by their greater need for vitamin A as compared with the control rats. One might further explain this increased requirement by supposing that the rancid fat, after its ingestion, effected a partial destruction of the vitamin A of the supplemental ration or even of the tissues. It would then be conceivable that the vitamin A actually supplied in this experiment was sufficient to cover the thus increased requirements of the female rats of the rancid series, but was somewhat insufficient to provide for the more rapid growth of which the male rats of the rancid series should have been capable.

But as such reasoning involves a number of rather improbable assumptions, and is highly speculative at best, we may well limit ourselves to observing that in this experiment, where the vitamin A was fed separately, the rancid lard, generally speaking, seemed to affect the growth and the general health of the rats receiving it less detrimentally than was the case in experiment 1, where the rancid lard came into intimate and prolonged contact with the vitamin A.

EXPERIMENT 3

Experiment 3 was a counterpart of experiment 1, except that the lard employed was the same as that used in experiment 2, and that only 8 rats were used instead of 12. The composition of the ration has already been given in connection with experiment 1, and the samples of rancid and sweet lard have been described in connection with experiment 2. The pertinent data concerning the experimental animals at the beginning of the experiment are recorded in Table V.

TABLE V.—*Description of rats used in experiment 3*

Rat No.	Ration	Sex	Initial age	Initial weight	Litter	Rat No.	Ration	Sex	Initial age	Initial weight	Litter
			Days	*Grams*					*Days*	*Grams*	
21	Sweet	Male	27	42	115–A	25	Rancid	Male	27	41	115–A
22	do	Female	27	42	115–A	26	do	Female	28	42	115–B
23	do	do	28	39	115–B	27	do	do	28	43	115–B
24	do	Male	27	39	115–A	28	do	Male	27	44	115–A

Throughout experiment 3 the rats receiving the rancid fat consumed less food and gained less weight than the corresponding controls. They were also less active and robust, and without exception devel-

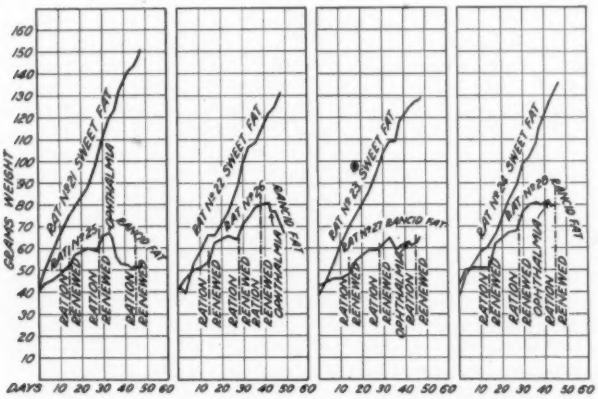

FIG. 3.—Growth curves of rats in experiment 3

oped pronounced cases of ophthalmia before the end of the experiment. In case of rat No. 25 of the rancid series, moreover, the hair remained scant and thin, the pink skin being clearly visible over the rump throughout the experiment. On the other hand, the rats of the rancid series manifested no definite symptoms of intestinal irritation as did the corresponding rats in experiment 1.

The smaller growth made by the rats in the rancid series as compared with the control rats is clearly shown by the growth curves in Figure 3. In case of the rats receiving the rancid fat, the subnormal growth, the development of ophthalmia, and the ultimate decrease in weight of rats Nos. 25, 26, and 28 bear clear testimony of a deficiency of vitamin A. From the data on food consumption presented

in Table VI, however, it is obvious that the rats of the rancid series consumed considerably less ration per day per gram of body weight than the corresponding controls; and we are left in doubt as to whether the deficiency was caused by a disappearance of vitamin A from the ration or by the limited food consumption. The average food consumption of the rats of the rancid series per day per gram of body weight, however, was 80 per cent of that of the control rats; and in view of the fact that the amount of egg yolk originally added to the ration was considerably in excess of that required for optimal growth of normal rats, we are inclined to believe that in spite of their limited food consumption the rats of the rancid series received enough egg yolk to have protected them from ophthalmia, if not to insure normal growth, had the vitamin A retained its potency. Therefore, while limited food consumption may have been, and probably was, a limiting factor in the growth of these rats, the evidence seems also to point to a loss of vitamin A from the ration containing the rancid lard.

TABLE VI.—*Food-consumption data for experiment 3, covering third to forty-eighth day, inclusive*

Ration	Rat No.	Sex	Total ration	Coefficient of food consumption *	Total gain
			Grams	*Grams*	*Grams*
Sweet	21	Male	371	0.0874	101
Do	24	do	324	.0856	94
Average for males			347	.0865	97
Sweet	22	Female	378	.1038	93
Do	23	do	320	.0806	85
Average for females			349	.0922	89
Average for males and females			348	.0893	93
Rancid	25	Male	180	.0740	10
Do	28	do	204	.0676	21
Average for males			192	.0708	15
Rancid	26	Female	223	.0762	21
Do	27	do	172	.0681	21
Average for females			197	.0721	21
Average for males and females			194	.0715	18

* See footnote to Table II.

This idea is further supported by the periodic nature of the growth curves of the rats in the rancid series. These curves have been marked at points corresponding to the introduction of freshly mixed ration, and it will be observed that the periods of accelerated growth invariably follow closely upon the renewal of the ration. While the records of food consumption are not sufficiently detailed to show whether or not the accelerated growth was accompanied by increased food consumption, it is clear that the ration containing the rancid lard, unlike the ration containing the sweet lard, deteriorated with increasing age; and the most plausible explanation of this fact would seem to be that the vitamin A of the ration was gradually destroyed by contact with the rancid fat.

This experiment, therefore, seems to confirm the inference already drawn from experiment 1, viz, that rancid fat apparently destroys

some of the vitamin A of food products with which it may be intimately mixed. It further suggests that the destruction of vitamin A, under the conditions here obtaining, proceeds gradually over a period of several days.

SUMMARY

Three experiments with rats bearing upon the characteristics of rancid lard as a food are reported. The lard used was more intensely rancid than would anywhere have been tolerated for human consumption, and probably more rancid than would be acceptable for animal feeding. It was also employed in larger proportions than those in which fat is generally used in the rations of man or beast. The results may be summarized as follows:

Under the conditions of these experiments, rancid lard, though clearly inferior to sweet lard as a ration component, did not seem to be actually toxic to the white rats receiving it.

The inferiority of rancid lard as a food product appeared to be due to its ability to destroy the vitamin A of the rations with which it was admixed. This destruction appeared to occur gradually over a period of several days, and was presumably due to the oxidation of the vitamin A by the organic peroxides of the rancid lard.

When the vitamin A was fed separately from the lard, the males receiving the rancid lard seemed to require more vitamin A than did males receiving a corresponding ration containing sweet lard.

THE LEAF-TISSUE FLUIDS OF EGYPTIAN COTTONS[1]

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INTRODUCTION

In 1921 a series of determinations of the physicochemical properties of the leaf-tissue fluids of Egyptian and Upland cottons and of their F_1 hybrid (*3*)[2] showed that, when grown under irrigation at Sacaton, in the Gila River Valley of southern Arizona, the leaf-tissue fluids of Pima Egyptian cotton are characterized by higher osmotic concentration, higher specific electrical conductivity, and higher hydrogen-ion concentration than those of Meade or Acala Upland cotton (*3*).

In subsequent studies it has been shown that the differentiation is also clearly marked in chloride (*4*) and in sulphate (*7*) content, and that the absorption of these two ions is differential (*5*).

The question will naturally arise, however, as to whether the sap properties found for Pima may be considered wholly typical of the Egyptian type in this regard. Kearney has shown (*8, 9, 10, 11*) that the Pima variety has a long history as an American-grown cotton. It seems quite possible that the differences between its tissue-fluid properties and those of the upland varieties with which it was compared may have arisen during this period in America.

The plausibility of this suggestion is emphasized by Balls' (*1*) conclusion that in Egypt the different strains of cotton differ in the salinity of their tissue fluids.

One purpose of the present investigation has been to determine whether varieties of Egyptian cotton other than Pima differ from Acala, Meade, and Lone Star Upland cotton in their tissue-fluid properties, or whether this difference is characteristic only of the American Egyptian variety, Pima.

MATERIALS AND METHODS

In 1922 plantings of seeds of five Egyptian varieties imported in that year from Egypt were made for comparison with Pima Egyptian and Meade upland cotton from American-grown seed. In 1924 the new

[1] Received for publication May 28, 1925; issued January, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 1033.

varieties were grown from seed which had been rendered more uniform by self-fertilization of plants selected as true to type. Three Upland varieties—Meade, Acala, and Lone Star—as well as Sea-Island cotton—were introduced in the comparison.

The exact cultural details need not be discussed here. The plantings were made in a manner similar to those adopted in earlier experiments. This involves the distribution of all of the varieties over the field in short subrows or subplots. Samples of mature leaf tissue were taken from all of the varieties of a subplot at the same time.

The leaf-tissue fluids were extracted and the physicochemical constants determined in a manner described in an earlier paper (*3*).

PRESENTATION AND ANALYSIS OF DATA

In the presentation of the results only the averages are given. Probable errors have been omitted for the following reasons: (*a*) The number of determinations on any one variety is small, and this renders the interpretation of the probable errors difficult under any conditions, particularly under the conditions of the present experiment; (*b*) the chief use of the probable errors of the means would have been in connection with the determination of the probable errors of the differences between any two varieties.

Such probable errors can be determined only if the correlation between the constants for the two varieties under comparison be known. The determination of such correlations on the basis of as large a number of varieties and as wide a spacing of varieties as are found in these experiments presents considerable difficulty.[3]

In the determination of the averages a somewhat different method has proved to be desirable for the two experiments.

While the plot utilized in the experiment of 1922 showed great irregularities of soil conditions, which were strikingly evident in the characteristics of the plants, a fairly uniform stand was obtained for the whole plot.

In the experiment made in 1924 the southern portion of the experimental plot carried a fairly uniform stand, but toward the northern end of the field many sections produced no plants at all. These differences in the salinity and texture of the soil, to which the diversities in stand are due, doubtless play an important part in determining the physicochemical properties of the plant-tissue fluids. It has seemed desirable, therefore, to prepare two sets of averages for the determinations made in 1924, the first comprising the southern end of the field, where materials could be obtained for all of the varieties, and the second including the northern end of the field, the stand there being not as good as that on the southern end. These two averages, with indications of the number of determinations on which these are based, are given as "partial" and "whole" series in the tables.

[3] It might seem that these difficulties should have been overcome by a different arrangement of the plantings. This, however, was impossible with the number of varieties involved in the present experiments without so reducing the number of plants per subplot that the determinations would have been based on tissues from one or but a very few individuals. In the organization of this experiment it seemed best, all things considered, to increase the size of the individual cultures, even though this resulted in the separation of the groups of plants to be compared.

The average values of osmotic concentration, as expressed in terms of freezing-point depression (Δ) in the various series of determinations made in the two years, are set forth in Table I. In the experiment of 1922, in both the first and the second series of determinations, all six of the Egyptian cottons show a greater freezing-point depression than the single variety (Meade) of Upland cotton.

TABLE I.—*Comparison of osmotic concentration in terms of freezing-point depression (Δ) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924 (E_1–E_5 are field key letters)*

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, E_1	8	1.275	8	1.285	8	1.233	8	1.166	12	1.243	14	1.230
Zagora, E_2	8	1.298	8	1.291	8	1.238	8	1.134	12	1.240	14	1.215
Sakel, E_3	8	1.345	8	1.378	8	1.371	8	1.374	12	1.379	15	1.444
Pelion, E_4	8	1.338	8	1.335	8	1.309	8	1.274	12	1.349	13	1.314
Assili, E_5	8	1.274	8	1.396	8	1.311	8	1.314	12	1.368	15	1.430
Pima	8	1.380	8	1.417	8	1.267	8	1.300	11	1.261	13	1.353
Sea island					8	1.151	8	1.134	8	1.151	11	1.152
Upland:												
Acala					8	1.112	8	1.061	8	1.112	11	1.078
Meade	8	1.251	8	1.247	8	1.147	8	1.086	8	1.147	11	1.146
Lone Star					7	1.106	8	1.037	7	1.106	11	1.102

In the series of determinations carried out in 1924 the averages based on the partial series and the entire series of determinations must be considered separately. In both the first and second partial series the freezing-point depression is higher in each of the six Egyptian varieties than in any one of the three different Upland varieties (Acala, Meade, and Lone Star).

In only one instance is the freezing-point depression of an Egyptian variety as low as that of Sea-Island cotton.

In both the first and the second whole series the averages of each of the six Egyptian varieties are numerically higher than those for the Upland varieties or for the Sea-Island cotton.

While the Egyptian varieties differ to some extent among themselves, taken as a class they are all characterized by distinctly higher osmotic concentration than the Upland varieties with which they are compared. These results show that higher osmotic concentration is not a peculiarity of the American Egyptian variety, Pima, but that it is characteristic of Egyptian varieties in general.

The experiments are not sufficiently extensive to justify final conclusions concerning the relative values of osmotic concentration in the Egyptian varieties themselves. It appears, however, that Ashmuni and Zagora have lower osmotic concentrations than the other four types, although in the first series of determinations Assili has about the same average freezing-point depression as the two just mentioned.

Turning now to the values of specific electrical conductivity as expressed in reciprocal ohms, we have the constants set forth in Table II. In all 12 comparisons which may be based upon the series of determinations made in 1922, the value of κ for the Egyptian type is higher than that for the single variety of the Upland type. In 1924 the same result is found for the comparison between the six Egyptian and the three upland types. Thus there can be no question that while the Egyptian varieties differ to some extent among themselves, they are, so far as investigated, characterized by higher specific electrical conductivity than the Upland types which have been grown under similar conditions in the investigations which have hitherto been possible.

TABLE II.—*Comparison of specific electrical conductivity* (κ) *in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924*

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, E_1	8	0.03282	8	0.03212	8	0.02949	8	0.02871	12	0.02936	14	0.02960
Zagora, E_2	8	.03255	8	.03170	8	.02977	8	.02776	12	.02970	14	.02878
Sakel, E_3	8	.03364	8	.03319	8	.03085	8	.03170	12	.03139	15	.03226
Pelion, E_4	8	.03463	8	.03307	8	.03149	8	.03135	12	.03223	13	.03231
Assili, E_5	8	.03288	8	.03199	8	.03000	8	.03070	12	.03109	15	.03208
Pima	8	.03449	8	.03339	8	.02948	8	.02991	11	.02878	13	.03034
Sea island					8	.02647	8	.02750	8	.02647	11	.02782
Upland:												
Acala					7	.02601	8	.02445	7	.02601	11	.02562
Meade	8	.03163	8	.03066	8	.02734	8	.02539	8	.02734	11	.02619
Lone Star					8	.02511	8	.02431	8	.02511	11	.02417

In both the first and second series of determinations of 1924, as epitomized in the averages for both the partial and the entire series, specific electrical conductivity for the Sea-Island cotton is lower than that of any of the Egyptian varieties. It may be higher or lower than that of the associated Upland cultures.

We now consider the ratio of specific electrical conductivity to freezing-point depression, κ/Δ. The averages are given in Table III. For the first series of determinations the average ratio for the six different Egyptian varieties is identical with that for the Upland type in so far as can be determined by the examination of averages not provided with probable errors. This result is in accord with the findings of an earlier investigation (*3*) on Pima Egyptian and Meade and Acala Upland cotton, in which the conclusion was drawn that there is no certain differentiation of the two types with respect to the ratio κ/Δ.

We now have to consider the concentration of two anions, for which absorption has been shown to be differential (*5*).

The results of an earlier investigation (*4*) have shown conclusively that Pima Egyptian cotton differs from Meade, Acala, and Lone Star Upland cotton in the chloride content of its leaf-tissue fluids.

Apparently Egyptian cotton has a greater capacity for the accumulation of chlorides with the march of the season (*6*) than has Upland cotton.

TABLE III.—*Comparison of the ratio of specific electrical conductivity (κ) to freezing-point depression (Δ) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924*

Variety	Determinations made in 1922				Determinations made in 1924							
	First series, Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni	8	0.02578	8	0.02506	8	0.02397	8	0.02461	12	0.02366	14	0.02421
Zagora	8	.02520	8	.02470	8	.02401	8	.02447	12	.02394	14	.02389
Sakel	8	.02512	8	.02426	8	.02254	8	.02311	12	.02278	15	.02250
Pelion	8	.02596	8	.02490	8	.02406	8	.02461	12	.02392	13	.02460
Assili	8	.02596	8	.02486	8	.02291	8	.02336	12	.02278	15	.02253
Pima	8	.02510	8	.02375	8	.02329	8	.02301	11	.02282	13	.02254
Sea island					8	.02304	8	.02425	8	.02304	11	.02417
Upland:												
Acala					7	.02348	8	.02360	7	.02348	11	.02334
Meade	8	.02537	8	.02473	8	.02390	8	.02340	8	.02390	11	.02296
Lone Star					8	.02261	8	.02291	8	.02261	11	.02246

The question naturally arises as to whether the higher chloride content is peculiar to the Pima variety of Egyptian cotton, which has developed in the Southwest from a long series of ancestors which had been grown under the frequently saline conditions of this region, or whether it is a characteristic common to Egyptian cottons in general. The results for a series of analyses carried out by a method proposed by Lawrence and Harris (*12*) appear in Table IV.

TABLE IV.—*Comparison of the chloride content (in terms of grams of Cl per liter) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1922 and 1924*

Variety	Determinations made in 1922				Determinations made in 1924							
	First series Aug. 10 to Aug. 13		Second series, Aug. 17 to Aug. 20		First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:												
Ashmuni, E_1	7	6.51	8	7.04	7	3.54	8	3.98	11	3.48	14	3.95
Zagora, E_2	8	6.14	8	6.82	8	3.56	8	3.66	11	3.56	14	4.04
Sakel, E_3	7	6.07	8	6.50	8	3.50	8	3.82	11	3.60	13	3.94
Pelion, E_4	7	6.86	8	6.95	8	4.12	7	4.73	12	4.30	11	4.82
Assili, E_5	8	6.21	8	6.59	7	3.16	7	4.20	11	3.79	13	4.50
Pima	7	7.44	8	7.89	7	3.02	8	3.59	10	3.05	13	3.53
Sea island					7	2.21	7	2.09	7	2.21	10	2.12
Upland:												
Acala					7	2.35	8	2.79	7	2.35	10	2.65
Meade	8	4.61	8	4.61	8	1.88	8	2.32	8	1.88	11	2.33
Lone Star					7	1.45	7	1.56	7	1.45	11	1.58

The averages for the determinations made in 1922 [4] show that all six of the Egyptian varieties have a higher chloride content than the Meade Upland cotton grown as a control, and that in both the first and second series of determinations the chloride content of the Pima Egyptian plants is slightly higher than that of any other Egyptian variety.

Because of certain analytical difficulties, the determinations based on the plants grown in 1924 must be regarded as only approximate. They indicate clearly, however, that all of the Egyptian varieties have a higher chloride content than either Sea-Island or the three Upland varieties included in this experiment.

In another place the writers have shown (*7*) that the sulphate content of the leaf-tissue of Pima Egyptian cotton is lower than that of the Meade or Lone Star varieties of upland cotton. Determinations on the sulphate content of other Egyptian varieties, made by the method of Gortner and Hoffman (*2*), are available for the experiment of 1924 only.[5] The determinations must be regarded as more or less approximate. They show clearly, however, that the sulphate content of the Upland varieties is higher than that of any of the Egyptian varieties considered, and higher than that of Sea-Island cotton.

TABLE V.—*Comparison of the sulphate content (in terms of grams of SO_4 per liter) in Egyptian, Sea-Island, and Upland cotton, as grown at Sacaton, Ariz., in 1924*

Variety	Determinations made in 1924							
	First partial series		Second partial series		First whole series		Second whole series	
	N	Mean	N	Mean	N	Mean	N	Mean
Egyptian:								
Ashmuni, E_1	8	10.14	8	8.58	12	10.44	13	8.93
Zagora, E_2	8	9.92	6	10.14	12	10.02	12	10.26
Sakel, E_3	8	10.15	8	10.01	12	10.41	14	10.19
Pelion, E_4	8	9.91	7	9.33	12	10.09	11	9.28
Assili, E_5	8	10.44	7	9.64	12	10.35	10	9.81
Pima	8	10.34	8	9.32	10	10.29	13	9.70
Sea-island	8	9.95	7	10.08	8	9.95	10	10.42
Upland:								
Acala	7	11.61	8	11.08	8	11.61	11	12.00
Meade	8	11.89	8	12.04	8	11.89	11	12.80
Lone Star	8	13.00	7	13.38	7	13.00	10	13.73

SUMMARY

The purpose of the present study has been to determine whether Pima Egyptian cotton, a variety of American origin, is unique in that it is different from at least some of the Upland varieties in the physico-chemical properties of the leaf tissue fluids as shown in earlier investigations (*3*, *4*, *5*, *6*, *7*), or whether all of the varieties of the Egyptian type differ from those of the Upland type of cotton.

The constants here considered represent, in addition to Pima, five Egyptian varieties grown from seed imported from Egypt in 1922. These are Ashmuni, Zagora, Sakel, Pelion, and Assili.

[4] The determinations of the chloride content of the samples of this series were made by Dr. and Mrs. John V. Lawrence.

[5] The analytical work on this series has been done by Clara T. Hoffman.

The results show that while the Egyptian varieties apparently differ among themselves, all of the six varieties here considered have a higher osmotic concentration and specific electrical conductivity than the upland varieties (Acala, Meade, and Lone Star) with which they have been compared. The two types apparently do not differ in the ratio of specific electrical conductivity to freezing-point depression.

All of the Egyptian forms considered have a higher chloride content and a lower sulphate content than the upland types. It may be recalled in this connection that Balls (*1*) concluded that the salt content of leaf tissues is specific in the varieties of cotton grown in Egypt.

It seems probable that differences between the individual varieties of the Egyptian type and between the individual varieties of the upland type may be demonstrated, but this will require more extensive and more refined experimentation for final proof. Constants for one series of sea-island cotton are given, but since other investigations on this type are under way the results will not be discussed in detail here.

LITERATURE CITED

(1) Balls, W. L.
1914. specific salinity in the cell sap of pure strains. Proc. Cambridge Phil. Soc. 17: 467–468.

(2) Gortner, R. A., and Hoffman, W. F.
1924. quantitative estimation of chlorides and sulphates in expressed plant tissue fluids. Bot. Gaz. 77: 96–102.

(3) Harris, J. A., Lawrence, Z. W., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T.
1924. the tissue fluids of egyptian and upland cottons and their F_1 hybrid. Jour. Agr. Research 27: 267–328, illus.

(4) ——— Lawrence, J. V., and Lawrence, Z. W.
1924. the chlorid content of the leaf tissue fluids of egyptian and upland cotton. Jour. Agr. Research 28: 695–704.

(5) ——— Hoffman, W. F., and Lawrence, J. V.
1925. differential absorption of anions by varieties of cotton. Proc. Soc. Expt. Biol. and Med. 22: 350–352.

(6) ———
1925. the accumulation of chlorides in the leaf tissue fluids of egyptian cotton with the march of the season. Proc. Soc. Expt. Biol. and Med. 22: 415–417.

(7) ——— Hoffman, W. F., and Hoffman, C. T.
the sulphate content of leaf tissue fluids of egyptian and upland cotton. Jour. Agr. Research 31: 653–662.

(8) Kearney, T. H.
1914. mutation in egyptian cotton. Jour. Agr. Research 2: 287–302, illus.

(9) ———
1921. heritable variations in an apparently uniform variety of cotton. Jour. Agr. Research 21: 227–242, illus.

(10) ———
1923. self-fertilization and cross-fertilization in pima cotton. U. S. Dept. Agr. Bul. 1134, 68 p., illus.

(11) ———
1923. segregation and correlation of characters in an upland-egyptian cotton hybrid. U. S. Dept. Agr., Bul. 1164, 57 p., illus.

(12) Lawrence, J. V., and Harris, J. A.
1924. tests of a wet oxidation and modified volhard method for the determination of chlorides in plant tissue fluids. Jour. Amer. Chem. Soc. 46: 1471–1477.

76649—26†——3

THE USE OF VACUUM FOR INSECT CONTROL [1]

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INTRODUCTION

The use of high vacuum for the control of insects seems never to have been considered seriously by the commercial world. The recent development and exploitation by private interests of a vacuum chamber intended for the treatment of certain warehoused commodities as a part of the equipment of a modern storage warehouse has aroused much interest in business circles and has given the writers an opportunity to make tests reported upon in this paper.

HISTORICAL

The possibility of killing insects by depriving them of air was conceived at least 250 years ago when the eminent scientist Robert Boyle (*2*)[2] invented a mechanical air pump [3] capable of exhausting a large proportion of the air from small receptacles. Boyle studied the effects of vacuums upon various forms of life. Unfortunately, from his published accounts one can not know the exact degree of vacuum employed. Although the vacuum obtained was by no means perfect, it proved effective in killing many forms with which he experimented. Boyle's experiments are of great interest, but lack of information makes it impossible to interpret them satisfactorily.

Jousset de Bellesme (*5, p. 710*), experimenting with vacuum in 1880, concluded that Hymenoptera could survive in vacuum for 5 or 6 hours at a temperature of 20° C. and Blattidae (cockroaches) for 2 hours at a temperature of 12° C.

Cole (*3, p. 69–71*), in 1906, in an account of the bionomics of the grain weevils, records experiments with vacuum upon *Sitophilus oryza*. According to his statement, adults of the rice weevil fed and oviposited when confined in a partial vacuum, and 5 out of the 10 weevils experimented with were alive at the end of 23 days. In the experiment the mercury of the gauge was reduced to 5 inches. In a second experiment, with the mercury reduced to 1 inch, 5 out of 10 adults were alive after an exposure to the vacuum for 15 days.

Nagel (*7*), in 1921, published an account of experiments conducted for the control of *Anobium striatum* Oliv. and reported that larvae of this pest were unharmed by exposure to vacuum for 24 hours.

The use of the vacuum in connection with fumigation work is well known and will not be discussed in this paper. Its use in connection with heat was tried by Mackie (*6*) for the control of the tobacco beetle infesting cigars. Mackie says (*6, p. 135*):

> The process in question consists of heating the tobacco to a certain degree after which the air is pumped out until a 28-inch vacuum is registered by the vacuometer. The material being heated higher than the vapor tension point

[1] Received for publication June 16, 1925; issued January, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 1041.
[3] The first mechanical air pump was invented by Otto von Guericke in 1654, an account of his experiments with vacuums having been published in 1672 (*4*). He experimented with birds and fish in vacuum but apparently did no work with insects. Boyle improved upon Von Guericke's pump and published an account of his experiments in 1670.

of water under this pressure, this causes the water content of all bodies comprehended therein to change to a gaseous form, and thus all insects are killed.

Brief notes on the effect on insects of a vacuum of 24 to 28 inches were given in a paper by the writers (*1*) at the Annual Convention of the American Warehousemen's Association, held in Chicago in December, 1924.

APPARATUS USED

The apparatus used by the writers in their experiments consisted of a concrete vault, 8 by 8 by 8 feet, and a large glass bell jar having a cubic content of approximately 825 cubic inches. The air was pumped from these two containers by means of a dry vacuum pump. In the case of the large concrete vault a vacuum of 28 inches was obtained within a period of approximately one and a half hours. An automatic control then cut off the motor, the pump remaining idle until the vacuum fell to 24 inches, when the control device automatically started the motor again. The small amount of leakage that occurred allowed the vacuum to fall from 28 inches to 24 inches in from 4 to 6 hours. In the case of the bell jar a 29-inch vacuum was obtained within 30 seconds of the time the pump was started, but little leakage occurred and no control device was used; whenever necessary, the pump was started by hand.

BELL-JAR EXPERIMENTS

EXPERIMENT NO. 1

In the first experiment specimens of 19 different species of insects affecting stored products were used. In some cases two or more stages of the insects were used, in others only the adults; in all cases from 10 to 50 specimens were used in each lot treated. The insects were subjected to a vacuum varying from 26 inches to 29 inches for periods of 1 day, 2 days, and 4 days, respectively. The temperature during the experimental period varied between 60° and 70° F. and the barometer reading for the period varied between 29.3 and 29.7 inches.

TABLE I.—*Effect of vacuum varying from 26 to 29 inches upon insects in bell jar of 825 cubic inches capacity*

Insect	Killed in 1 day				Killed in 2 days				Killed in 4 days			
	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Alphitobius piceus Oliv.	------	------	------	100	------	100	------	100	------	100	------	100
Anthrenus fasciatus Hbst.	------	50	100	100	------	100	100	100	------	100	100	100
Attagenus piceus Oliv.	------	50	------	100	------	100	100	100	------	100	100	100
Cryptolestes pusillus Schon	------	------	------	100	------	100	------	100	------	100	100	100
Dermestes vulpinus Fab.	------	100	------	100	------	100	------	100	------	100	100	100
Ephestia kuehniella Zell.	------	100	100	100	------	100	100	100	------	100	100	100
Necrobia rufipes De Geer	------	------	------	100	------	------	------	100	------	------	------	100
Oryzaephilus surinamensis L.	------	------	------	100	------	------	------	100	------	100	------	100
Plodia interpunctella Hbn.	------	100	100	100	------	100	------	100	------	100	100	100
Silvanus gemellatus Duv.	------	------	------	100	------	------	------	100	------	100	100	100
Sitophilus oryza L.	------	------	------	100	------	------	------	100	------	------	------	100
Sitophilus granarius L.	------	------	------	100	------	------	------	100	------	------	------	100
Tenebrio obscurus Fab.	------	------	------	100	------	------	------	100	------	100	------	100
Tenebroides mauritanicus L.	------	80	------	50	------	100	------	100	------	100	------	100
Tineola biselliella Hum.	10	50	100	100	40	100	100	100	100	100	100	100
Tinea pellionella L.	------	40	------	------	------	100	------	------	------	100	------	------
Tribolium confusum Duv.	------	------	------	100	------	------	------	100	------	100	100	100
Tribolium ferrugineum Fab	------	------	------	100	------	------	------	100	------	100	100	100
Trogoderma tarsale Melsh	------	0	------	------	------	20	------	------	------	100	100	100

As indicated by the data of Table I, an exposure of 24 hours was fatal to nearly all the insects treated. Larvae of the dermestid beetles proved quite resistant, only 50 per cent of those of *Anthrenus fasciatus* and *Attagenus piceus*, and none of *Trogoderma tarsale* being killed. The eggs of the webbing clothes moth, *Tineola biselliella*, and the larvae of both this clothes moth and the case-making clothes moth, *Tinea pellionella*, showed much resistance.

A two-day exposure killed all specimens treated with the exception of the eggs of *Tineola biselliella*, of which only 40 per cent were killed, and the larvae of *Trogoderma tarsale*, of which only 20 per cent were killed. A four-day exposure killed all stages of all insects.

EXPERIMENT NO. 2

In the second experiment the same species of insect pests were used, except that *Gnathocerus cornutus* and *G. maxillosus* displaced *Sitophilus granarius*. A 29-inch vacuum was obtained and the vacuum was not allowed to fall below 28 inches at any time during the course of the experiment. The barometer reading was 29.3 inches and the temperature for the period varied between 60° and 70° F.

As shown in Table II, a large percentage of the insects treated were killed by a seven-hour exposure. Adults of all species were killed, but the larvae of Tenebrio, Tineola, Tinea, and Trogoderma were apparently but little affected by the seven-hour exposure, whereas 70 per cent of larvae of Ephestia and 80 per cent of larvae of Plodia were killed. A one-day exposure to this vacuum killed all stages of all insects experimented with, with the exception of the larvae of *Trogoderma tarsale*, 50 per cent only of which were killed, and the eggs of *Tineola biselliella*, 90 per cent of which survived.

TABLE II.—*Effect of vacuum varying from 28 to 29 inches upon insects in bell jar of 825 cubic inches capacity*

Insect	Killed in seven hours			Killed in one day			
	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Alphitobius piceus Oliv			100				100
Anthrenus fasciatus Hbst			100		100	100	100
Attagenus piceus Oliv	100	100	100		100	100	100
Cryptolestes pusillus Schon			100		100	100	100
Dermestes vulpinus Fab	100		100		100		100
Ephestia kuehniella Zell	70		100		100	100	100
Gnathocerus cornutus Fab			100				100
Gnathocerus maxillosus Fab			100				100
Necrobia rufipes De Geer			100				100
Oryzaephilus surinamensis L			100				100
Plodia interpunctella Hbn	80		100		100	100	100
Silvanus gemellatus Duv			100				100
Sitophilus oryza L			100				100
Tenebrio obscurus Fab	0		100				100
Tenebroides mauritanicus L					100		100
Tineola biselliella Hum	0			10	100	100	100
Tinea pellionella L	0				100		
Tribolium confusum Duv			100				100
Tribolium ferrugineum Fab			100				100
Trogoderma tarsale Melsh	0				50		

VAULT EXPERIMENTS

Specimens of 20 species of insects were used in the experiments conducted in the concrete vault. They were confined in pill boxes or glass vials stoppered with cotton, and individuals of each species were placed on a ledge of an observation window so that their actions could be observed at all times. A vacuum of from 24 inches to 28 inches was maintained automatically throughout the experiment. The barometer reading for the period varied between 29.3 and 29.7 inches; the temperature varied between 60° and 70° F. At the end of three days specimens of all species of insects were removed and examined.[4] Other specimens of all species were removed at the end of 4 days, 5 days, 6 days, and 7 days, respectively.

The data in Table III indicate that exposure to a vacuum varying from 24 to 28 inches for three days killed a large proportion of the insects. A very considerable number of the coleopterous larvae survived, only 20 per cent of those of *Trogoderma tarsale* being killed, and 60 per cent of those of *Tenebrio obscurus*. The softer-bodied larvae of *Dermestes vulpinus* and the lepidopterous larvae were killed. The adults which escaped belong to the small grain-infesting species, with the exception of adults of Alphitobius which are larger by comparison.

TABLE III.—*Effect of vacuum varying from 24 to 28 inches upon insects in a concrete vault containing 512 cubic feet*

Insect	Killed in three days				Killed in four days				Killed in five days				Killed in six days				Killed in seven days			
	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults	Eggs	Larvae	Pupae	Adults
	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
Alphitobius piceus Oliv	---	90	---	86	---	90	---	86	---	100	---	98	---	100	---	98	---	100	---	100
Anthrenus fasciatus Hbst	---	92	100	100	---	100	100	100	---	100	100	100	---	100	100	100	---	100	100	100
Attagenus piceus Oliv	---	66	100	100	---	78	100	100	---	78	100	100	---	80	100	100	---	95	100	100
Cryptolestes pusillus Schon	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Dermestes vulpinus Fab	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100	---	100
Ephestia kuehniella Zell	---	100	100	100	---	100	100	100	---	100	100	100	---	100	---	100	---	100	---	100
Gnathocerus cornutus Fab	---	90	---	98	---	100	---	100	---	100	---	100	---	100	100	100	---	100	100	100
Gnathocerus maxillosus Fab	---	90	---	94	---	100	---	100	---	100	---	100	---	---	---	100	---	---	---	100
Necrobia rufipes De Geer	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Oryzaephilus surinamensis L	---	---	---	50	---	---	---	65	---	---	---	75	---	---	---	90	---	---	---	100
Plodia interpunctella Hbn	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Silvanus gemellatus Duv	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Sitophilus oryza L	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Sitophilus granarius L	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Tenebrio obscurus Fab	---	60	---	100	---	60	---	100	---	80	---	100	---	80	---	100	---	80	---	100
Tineola biselliella Hum	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Tinea pellionella L	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100	---	---
Tribolium confusum Duv	---	---	---	98	---	---	---	99	---	---	---	100	---	---	---	100	---	---	---	100
Tribolium ferrugineum Fab	---	---	---	99	---	---	---	100	---	---	---	100	---	---	---	100	---	---	---	100
Trogodema tarsale Melsh	---	20	---	---	---	20	---	---	---	20	---	---	---	30	---	---	---	30	---	---

The data indicate that exposure for 4, 5, 6, and 7 days did not kill all individuals. It is interesting to note that adults of *Alphitobius piceus* and *Oryzaephilus surinamensis* were not all killed by an exposure of 6 days (although the last ones succumbed on the seventh

[4] All treated specimens were held for two weeks after the experiment and were examined at frequent intervals to see if any would recover.

day), and that 7 days' exposure did not kill all larvae of *Attagenus piceus*, *Tenebrio obscurus*, and *Trogoderma tarsale*.

Additional experiments, in which specimens of these insects were exposed to the same vacuum for two days only, gave the following results: Larvae of *Plodia interpunctella*, *Ephestia kuehniella*, and *Pyralis farinalis*, and adults of *Sitophilus oryza* and *S. granarius* were all killed. Many larvae and adults of the clothes moth *Tineola biselliella* were killed, but specimens of the other insects were apparently little affected.

GENERAL OBSERVATIONS

By using the glass bell jar and the observation windows of the vault the activity of the insects in the vacuum chamber could be readily observed. It was noted that when the insects were subjected to a 29-inch vacuum, all movement ceased in all stages of all species within two minutes after the pump was started. Beetles and moths fell upon their backs with every appearance of being dead. If the air was restored within a short time the insects regained their activity and appeared little the worse for their experience, except in the case of adults of the meal worm *Tenebrio obscurus*. The meal-worm beetles when treated to this vacuum for only 10 minutes showed feeble movements after being restored to the air, but died within a few hours thereafter.

When insects were exposed to the 24 to 28 inch vacuum many became inactive soon after the vacuum was obtained but others remained slightly active for several days.

In general, the adult and pupal stages of the insects experimented with were more susceptible to the effect of vacuum than were their larval stages. Lepidopterous insects were for the most part rather easily killed. Treated specimens of the larvae of these and other insects were stiff and brittle and appeared to have been thoroughly dried out by the process. To test the effect of vacuum on water, a small amount was placed in a glass vial under the bell jar and subjected to a 29-inch vacuum; the water boiled violently until the air had been removed from it.

Candy, which is often infested with insects, was also treated and the effect noted. Chocolate creams were broken open by the force of the vacuum, although chocolate-covered nuts were apparently uninjured.

Insects sealed in cartons of breakfast foods and buried in rolls of clothing or in upholstered furniture were killed as quickly as though they had been exposed in pill boxes or cotton-stoppered glass vials.

SUMMARY

A vacuum of 28 to 29 inches, maintained for 7 hours in a bell jar of 825 cubic inches capacity when the temperature ranged from 60° to 70° F. and the barometer read 29.3 inches, killed all adults of *Alphitobius piceus*, *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Gnathocerus cornutus*, *G. maxillosus*, *Necrobia rufipes*, *Oryzaephilus surinamensis*, *Plodia interpunctella*, *Silvanus gemellatus*, *Sitophilus oryza*, *Tenebrio obscurus*, *Tribolium confusum*, and *T. ferrugineum*. It killed all pupae of

Attagenus piceus, and all larvae of *A. piceus* and *Dermestes vulpinus*, 70 per cent of the larvae of *Ephestia kuehniella* and 80 per cent of the larvae of *Plodia interpunctella*. Larvae of *Tenebrio obscurus*, *Tineola biselliella*, *Tinea pellionella*, and *Trogoderma tarsale* were apparently not affected. Exposure for 24 hours to this vacuum killed all adults, pupae, and larvae except 50 per cent of the larvae of *Trogoderma tarsale*. Eggs of none of the species were to be had except of *Tineola biselliella*, and of these only 10 per cent were killed.

A vacuum of 26 to 29 inches in the glass bell jar, with the temperature ranging from 60° to 70° F., and the barometer from 29.3 to 29.7 inches, maintained for four days, killed all adults of *Alphitobius piceus*, *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Necrobia rufipes*, *Oryzaephilus surinamensis*, *Plodia interpunctella*, *Silvanus gemellatus*, *Sitophilus oryza*, *S. granarius*, *Tenebrio obscurus*, *Tenebroides mauritanicus*, *Tineola biselliella*, *Tribolium confusum*, *T. ferrugineum*, and *Trogoderma tarsale*. It killed all pupae used in the experiment, including those of *Anthrenus fasciatus*, *Attagenus piceus*, *Cryptolestes pusillus*, *Dermestes vulpinus*, *Ephestia kuehniella*, *Plodia interpunctella*, *Silvanus gemellatus*, *Tineola biselliella*, *Tribolium confusum*, *T. ferrugineum*, and *Trogoderma tarsale*. All larvae of the 18 species above mentioned except Necrobia and Sitophilus (which were not included in the experiment) and the larvae of *Tinea pellionella* were killed, and all the eggs of *Tineola biselliella*. Eggs of this species were the only eggs available for experimental purposes.

Exposure of two days to the 26 to 29 inch vacuum killed all forms included in the experiments except 80 per cent of the larvae of *Trogoderma tarsale* and 60 per cent of the eggs of *Tineola biselliella*. Exposure for only one day killed a large proportion of the forms included except 50 per cent of the adults of *Tenebroides mauritanicus*, 50 per cent of the larvae of *Anthrenus fasciatus*, *Attagenus piceus*, and *Tineola biselliella*, 40 per cent of the larvae of *Tinea pellionella*, 80 per cent of the larvae of *Tenebroides mauritanicus*, none of *Trogoderma tarsale*, and only 10 per cent of the eggs of *Tineola biselliella*.

A vacuum of 24 to 28 inches, maintained in a concrete vault 8 by 8 by 8 feet, when the temperature varied between 60° and 70° F., and the barometer readings were between 29.3 and 29.7 inches, gave results which indicate that the usual fabric pests troublesome in storage warehouses can be killed by the vacuum treatment. Eggs, larvae, pupae, and adults of the common or webbing clothes moth, *Tineola biselliella*, were dead at the end of exposures of 3, 4, 5, 6, and 7 days. All pupae and adults of the carpet beetles *Attagenus piceus* and *Anthrenus fasciatus* were dead at the end of the same exposures. Ninety-two per cent of the larvae of *Anthrenus fasciatus* were killed by the end of an exposure of 3 days, and all after 4, 5, 6, and 7 days. The larvae of *Attagenus piceus* were most resistant; only 66 per cent being killed by an exposure of 3 days, 78 per cent by exposures of 4 and 5 days, 80 per cent by an exposure of 6 days, and 95 per cent by an exposure of 7 days. *Oryzaephilus surinamensis* was the most resistant of all species tested in the adult stage, 50, 65, 75, 90, and 100 per cent being killed by exposures of 3, 4, 5, 6, and 7 days, respectively. The larvae of *Attagenus piceus*, *Tenebrio*

obscurus, and *Trogoderma tarsale* were the most resistant of the larval forms used, 3 days' exposure killing only 66, 60, and 20 per cent, respectively; and 7 days' exposure killing 95, 80, and 30 per cent, respectively.

LITERATURE CITED

(1) BACK, E. A., and COTTON, R. T.
[1925]. COMMON PESTS DESTRUCTIVE TO HOUSEHOLD GOODS IN STORAGE. Proc. Amer. Warehousemen's Assoc. (1924) 34: 185–188, illus.

(2) BOYLE, R.
1670. NEW PNEUMATICAL EXPERIMENTS ABOUT RESPIRATION. Phil. Trans. Roy. Soc., London 5 (62): 2011–2031; (63): 2035–2056.

(3) COLE, F. J.
1906. THE BIONOMICS OF GRAIN WEEVILS. Jour. Econ. Biol. (1905/06) 1: 63–71.

(4) GUERICKE, O. VON
1672. EXPERIMENTA NOVA (UT VOCANTUR) MAGDEBURGICA DE VACUO SPATIO. 244 p., illus. Amstelodami.

(5) JOUSSET DE BELLESME, [G. L. M. F.]
1881. REMARQUES SUR LA RESPIRATION DES INSECTES. Assoc. Franç. Avanc. Sci. Compt. Rend. (1880) 9: 710–712.

(6) MACKIE, D. B.
1917. A SUMMARY OF THE WORK OF THE PEST CONTROL SECTION FOR THE YEAR 1916. Philippine Agr. Rev. 10: 128–145, illus.

(7) NAGEL, W.
1921. BEKÄMPFUNG VON ANOBIUM STRIATUM OLIV. MITTELS CYANWASSERSTOFFGASEN. Ztschr. Angew. Ent. 7: 340–348.

76649—26†——4

THE RELATION OF SIZE OF KERNELS IN SWEET CORN TO EVENNESS OF MATURITY [1]

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INTRODUCTION

Commercial canners have found a wide range in the degree of maturity of sweet-corn ears in the canning season. When the majority of the ears are ready to harvest some have already become too hard and tough, and others are still too young and watery to give the pack the proper consistency. This unevenness of maturity varies with the variety and appears to be worst in the small or narrow grain types. It is particularly troublesome in the Country Gentleman, where the slender "shoe-peg" kernel predominates. It causes a lack of uniformity in the canning condition of the corn, which results in a pack of poor quality unless the ears are properly graded, an operation which adds to the expense of handling the crop since the grading must be done by hand. In order that these expensive variations may be avoided, it is important that as much of the crop in each field mature at the same time as possible.

In 1918 the Indiana Canners' Association requested the horticultural department of the Purdue station to study the sweet-corn problem with a view to developing better strains of the standard canning varieties and ones that would be more suitable to Indiana conditions. The work reported here is a part of this larger problem.

For several years, while sweet-corn seed was being tested for germination and freedom from disease, it was observed that the small kernels produced slenderer sprouts with smaller root systems than the larger kernels. This was true whether the kernels were picked from the same ears or from bulk-shelled samples. The question then arose as to whether or not this condition affected the rate of growth and time of maturity of the progeny. In the fall of 1920 large and small kernels of the same varieties were planted in the greenhouse to determine whether the differences noted in the germinator would be found in soil-grown plants. Very striking differences both in size of seedlings and in rate of growth developed, and the investigation was enlarged and continued for more than two years. During this time a large number of observations and experiments were made under greenhouse and field conditions, and these will be discussed in the following pages.

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[2] The writer wishes to thank the Fame Canning Co., of Shelbyville, Ind., for helpful cooperation in some of the investigations here reported.

REVIEW OF LITERATURE

Investigators working with a great many varieties have found that for the most part large, heavy seeds produce plants of greater size and higher yield than small ones, and the crop is more uniform. Other investigators, however, have found the results of their work conflicting or inconclusive. A brief résumé of published data is given here in order that the scope of the investigations may be understood, but it is not intended to include all that have been reported.

Dehérain and Dupont (*3*)[3] claim that a definite advantage in favor of heavier seed shows only when the difference in weight is great. Myers (*10*), working with wheat, found his results inconclusive. Leighty (*8*) criticizes the method of selecting large seed without knowing something of the characteristics of the mother plant. Love (*9*), in his work with wheat and oats, finds that the heaviest grains come from the tallest and heaviest yielding plants. Johannsen (*5*), from his studies of the inheritance of weight of bean seeds, concludes that the heaviest daughter beans are the progeny of the heaviest mother beans, but that this does not necessarily hold in a pure line. DeVries (*15*), on the other hand, thinks that size and weight of seeds are the result of nutrition in the broad sense rather than of inheritance.

Studies have also been made of seeds in relation to their specific gravity. Haberlandt's (*4*) work with wheat and oats shows that the denser kernels yield heavier returns in grain and the lighter kernels yield greater quantities of straw. Wollny (*16*) maintains that its absolute weight and not its specific gravity is the true index to the value of grain. Clark (*1*) finds that size of seed is the most important factor for selecting within a variety, that is, the heaviest seed in a given size (volume) gives the best results.

In studying the combined effects of size and specific gravity, Sanborn (*14*) found that the yield from the lighter grains of wheat is greater than from the heavier ones. Degrully (*2*), working with corn, discarded all of the very small and poorly formed kernels and separated out the lightest one-fourth of the remainder by means of sodium nitrate solution. The difference in results in favor of the heavy kernels was very striking. He also found that wheat seedlings from heavy grains are greener, more vigorous, and greatly superior to those from lighter grains. These differences, he believes, are the result of some factor inherent within the seed. Kiesselbach and Helm (*6*) found that the total "sprout values" of unselected grains and large and small grains of wheat were, respectively, 100, 123, and 100.88. These figures indicate a rather close relationship between the size of seed and its sprout value. Kiesselbach and Cook (*7*) working with corn find that increases in kernel weight may be due to two causes: (1) heterosis (11.8 per cent) as in crosses in selfed strains, and (2) a change in type of endosperm (19.9 per cent), as sweet corn crossed with dent or flint varieties. Commercial dent varieties, being already heterozygous, respond relatively little (0.2 per cent increase) to the immediate effect of foreign pollen as a result of heterosis. Pearl and Surface (*11*), working with corn, found that there is a marked tendency for the plants which were relatively small

[3] Reference is made by number (italic) to "Literature cited," page 1052.

at the beginning of the season to remain, on an average, relatively small throughout most of the season; extreme types at the beginning of the season tend strongly to remain extreme types during the whole season. This behavior is thought to be the result of internal rather than external causes. Reed (*12*) shows that the seedlings of Helianthus follow the same tendency as that described by Pearl and Surface for corn. Plants which were small at the beginning were generally small at maturity; those which were large at the beginning usually remained so throughout the season. He also concluded that this behavior is the result of inherent tendencies rather than of external causes. In studying garden beans Renich (*13*) found that large, heavy seeds give the best growth and the largest plants. The range of variation in size and weight of seedlings is wider between those from small and medium-sized seeds than between the medium-sized and large seeds.

MATERIALS AND METHODS

Since Country Gentleman is one of the varieties of sweet corn that varies most widely in the size of its kernels, it was chosen for most of the experiments, although other varieties were used. An examination of the kernels showed that on the same ear they varied in size from the very slender "shoe-peg" type to the larger and broader crowned types (pl. 1, A). These large and small types of kernels gave, respectively, large and small seedlings in the germinator with correspondingly large and small root systems. Disease-free seed was used, and it was certain that these differences were the result of natural causes and in no way attributable to disease.

GREENHOUSE TESTS

In order that their behavior in the soil might be observed, the kernels of each type were planted in the greenhouse early in the spring of 1921 before the weather was warm enough for field planting (pl. 1, B). No difference was noted in the time required for the two types to germinate and the seedlings to appear. During the first few days the seedlings grew rapidly, but even at this time marked differences were evident between the two sets of progeny; those from the small kernels were slender with narrow leaves, while those from the large kernels were stocky and vigorous and their leaves were wide.

The greenhouse tests were repeated in the spring of 1922, a larger number of kernels of each grade being used than had previously been employed. These experiments were made with the double purpose of determining the rate of germination of the two grades and the relative size of the progeny. The kernels of both types germinated at about the same time, and both varied somewhat, but neither had a distinct advantage over the other. Representative stalks of both types were transplanted to the field as soon as weather conditions would permit the making of careful growth studies. The data for these stalks are presented later in the paper.

FIELD TESTS

In 1921 a large number of kernels of both types were picked out by hand and kept separate for use in field tests. A part of the samples were taken from individual ears and the rest from a shelled bulk

sample. Where the samples were picked from the ears the kernels were taken from all parts, so that they would represent the whole ear rather than separate portions of it. Those samples taken from the same ears were planted in parallel rows, adjacent to each other (pl. 1, C). A test of 13 ears was included in this experiment, the results of which are shown in Table I.

TABLE I.—*Field performance of large and small kernels taken from the same ears of sweet corn*

Row No.	Ear No.	Kernel grade	Number of kernels planted	Percentage stand	Relative size of stalks	Days to full tassel	Stage of sweet corn
1	1	Large	28	85.7	Large	47	Canning.
2		Small	27	40.7	Small	54	Premilk.
3	2	Large	30	76.6	Large	51	Canning.
4		Small	29	37.9	Small	54	Premilk.
5	3	Large	31	96.8	Large	51	Canning.
6		Small	27	70.4	Small	56	Premilk.
7	4	Large	28	92.8	Large	51	Canning.
8		Small	26	76.9	Small	54	Premilk+[a].
9	5	Large	27	96.7	Large	51	Canning+.
10		Small	27	77.7	Small	56	Premilk+.
11	[b]6	Large	29	93.1	Large	51	Canning.
12		Small	28	85.7	Medium	54	Premilk+.
13	[b]7	Large	27	92.6	Large	47	Canning+.
14		Small	30	86.6	Medium	50	Canning.
15	8	Large	30	93.1	Large	54	Canning.
16		Small	27	90.0	Small	57	Premilk+.
17	9	Large	30	96.6	Large	47	Dough−.
18		Small	27	85.2	Small	55	Canning−.
19	10	Large	25	80.0	Large	51	Canning.
20		Small	29	96.5	Small	54	Canning−.
21	11	Large	25	84.0	Large	47	Canning.
22		Small	27	85.2	Small	51	Premilk.
23	[b]12	Large	28	92.8	Large	47	Dough−.
24		Small	24	91.7	Medium	47	Canning.
25	13	Large	22	95.4	Large	47	Canning.
26		Small	22	72.2	Small	53	Premilk.

[a] The plus and minus signs are used to indicate conditions of maturity "greater than" or "less than," respectively, the state of maturity with which they are used. Thus "premilk +" means that the corn is slightly past the premilk stage, but not yet in the best canning condition, as determined by the thumbnail test.

[b] The kernels in the "small" grade were distinctly smaller than those in the "large" grade, but neither as slender nor as light as those from the other ears; in a bulk sample they would be classed as medium-sized. Consequently, the stalks from these kernels were less delayed in beginning their growth, and there was less difference in time of reaching the canning stage.

The most significant data in Table I are those for percentage of stand, days of full tassel, relative size of stalks, and condition of the corn when the ears on the large stalks were ready to pick. In studying the stand or germination of the seeds for the various ears and grades it was found that the large kernels germinated better than the small ones. The large seed produced larger and stockier plants than the small ones, and the greater the difference in the size of the kernels the greater the difference in the size of the plant. These differences were plainly evident from the beginning and remained so until the stalks were mature. The large stalks reached full tassel about five days before the small ones and the ears reached the canning stage with about the same time interval. When the ears of the large stalks were in the best canning condition, those on the small stalks were so tender and watery that the corn was unfit for use, and it would have taken about five days longer under the existing conditions for it to reach the same stage.

A.—Large and small kernels of Country Gentleman sweet corn. These sizes are found in all parts of the same ear and are very common in this variety.

B.—Stalks produced by the kernels shown in A. The large stalks on the right were produced by the large kernels. The small ones in the middle were produced by the small kernels, and those on the left by a mixture of both types. Note the small stalks in the foreground and the large ones in the rear of this row.

C.—Parallel rows planted with large and small kernels from the same ears. Rows 1 and 2 (left to right) are from ear No. 1, and rows 3 and 4 are from ear No. 2, the data for which are given in Table I. Note the correlation between size of kernels and size of stalks.

Another field test was made at Shelbyville, Ind., in 1922, in cooperation with a commercial canning company. This test was conducted on a larger scale and bulk seed was used instead of that selected from individual ears. The corn used was a strain of Country Gentleman which had been grown and selected under local conditions for four years. It had become fairly well acclimated in this time and was of good type for the variety. A sufficient number of large and small kernels were hand picked to plant a half acre each, and in addition to these plots one of equal size was planted with ungraded seed from the same source to serve as a basis for comparison.

The soil was a very rich, uniform, black loam. It was well drained and in a fine state of cultivation. The corn was drilled with a two-horse corn planter, the smallest plates being used.

When the corn came up the stand was very good. The stalks in the plot planted with large kernals were uniform in vigor, size, and color. There were practically no small or slender stalks in the whole plot; they were vigorous and stocky, and dark green in color.

The stalks in the plot planted with small seed were much shorter and slenderer than those from the large kernels. They were fairly uniform in color, but varied somewhat in size. (Note the similarity of the following observations to the data given in Table III.)

A wide range of variation was evident in the plot planted with the ungraded bulk seed. The stalks ranged in size from the small, slender types to the large, stocky ones, with various intermediate types between.

At tasseling time the stalks from the large kernels came into full tassel evenly and about five days in advance of those from the small ones; in fact, it was possible to distinguish these rows a long distance from the field by this earlier and more uniform development. The stalks from the small kernels were distinctly smaller and later at this time; they were tasseling irregularly and only about 20 per cent had reached the tassel stage.

The plot planted with the ungraded seed was also very irregular. It was composed of a mixture of stalks varying in size and maturity from those in full tassel to those in which the tassels were just beginning to form.

HARVEST DATA

Outstanding differences were noted in the plots at harvest time. These consisted chiefly in time and evenness of maturity and size of stalks and yields. The plants from the large seeds were larger and more uniform in maturity than any in the series, about 95 per cent having reached maturity at the same time. They were well developed and the kernels were of fair size and deep. A good many of the stalks bore two full-sized ears.

The stalks from the small kernels were shorter and slenderer than those from the large ones and were uneven in maturity. A few were as mature as those in the plot from the large seed, some had quite recently come into full silk, but the majority were just passing out of the premilk stage, that is to say, they were about five days later than the plants from the large seed.

The plot planted with ungraded seed was also very irregular in the size of its stalks and the maturity of its ears. The latter ranged

from the canning stage through all other stages down to the fresh silk. Two pickings were made in each plot. Nearly all of the corn was removed the first time from the plot planted with large kernels, but about half of the yield of the other two plots remained for the second picking.

TABLE II.—*Field performance of large and small kernels from the same bulk sample*

Plot	Number of bearing stalks	Percentage of barrenness	Yield	Average yield per plant
			Tons per acre	
Ungraded kernels	2,775	16	2.40	1.70
Large kernels	2,406	8	2.53	2.14
Small kernels	3,019	25	2.63	1.60

Table II gives a summary of the field performance of the three plots. The total stand varied with the size of the seed because the planter was unable to drop the small kernels as evenly as the larger ones. It will be noted that the percentage of barren and unproductive stalks was smallest among the large kernels, largest among the small ones, and intermediate in the plot of ungraded seed. The yield per acre was greatest in the plot planted with small kernels owing to the greater number of plants. The average yield per plant also varies with the size of the seed. The large kernels yielded 0.54 pound per plant more than the small kernels and 0.44 pound more than the ungraded seed. The average yield for the ungraded seed was higher than that for the small seed because of the large kernels contained in it. Many of these kernels produced two ears, just as did those in the plot planted with large seed, a fact which tended to raise the average yield per stalk.

PLANT-GROWTH MEASUREMENTS

In order to obtain detailed information on the relative rate of growth of cornstalks from large and small kernels, the following data were carefully collected. Thirty representative stalks from both types of kernels were selected from a large number which were started in the greenhouse. These were transplanted to the field and records of growth were taken at weekly intervals throughout their development. The data consisted in height of stalk, diameter of base, time of tasseling, time of shedding pollen, time of appearance of silk, time of arrival at full silk, time at which the ear reached the canning stage, yield of plant, size of ears, size of kernels, and number of tillers (suckers) for each type of plant. As many of these data as possible are presented in Figure 1, some are given in Table III, and the remainder are discussed in the following paragraphs.

Figure 1 shows the rate of growth, time of tasseling, final height, period of pollen shedding, time at which the ears came into full silk, and time at which they reached the canning stage. It so happened that transplanting was done at the beginning of a droughty period, and as long as the drought continued the plants made little growth.

The large stalks suffered less than the small ones and the line of growth shows a greater angle of elevation; this period is represented by the flattened portion of the graph. The lines of growth separate immediately after the corn comes up and they never again unite. Large seeds have more reserve food material than small ones, and this seems to be the factor which determines the difference in the initial growth. The germinator showed, as previously stated, that the root systems of the plants from the large kernels were much larger than from the small ones and these provide a means of supplying the plants with greater quantities of food materials from the soil. It is believed that these two factors are responsible for the difference in growth and time of development in the two types.

Figure 1 shows also that there is a difference of five days in tasseling, silking, and reaching the canning stage between corn grown from large and corn grown from small kernels, a fact which accounts for

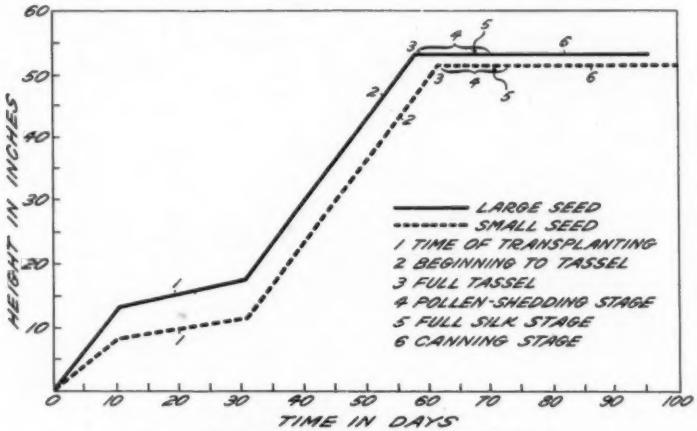

FIG. 1.—The relation of kernel size to evenness of maturity

the unevenness of maturity found in fields of sweet corn grown for canning purposes.

The height of the plant was measured by taking the highest point reached when the leaves were held upright, except the final measurement, which was taken from the tip of the tassel when it had stopped growing. The diameter of the stalks at the base was taken in the middle of the first internode and shows a constant enlargement in proportion to the height of the plant.

Table III gives a part of the data collected in the plant growth studies which could not be expressed in the preceding graph. The results reported in the table are very consistent and substantiate the observations made at Shelbyville, Ind., the same year, and also the results obtained at the station the previous year. At 9 days of age the plants from the large seed averaged 4.15 inches taller and 0.048 inch greater in diameter than the average from the small seed and 2.05 inches taller and 0.023 inch greater in diameter than the average from the ungraded seed.

At maturity, after all growth had stopped, there was still some difference in size between the stalks of the different grades, although not so much as there had been earlier in the season. The stalks from the large seed averaged 1.6 inches taller and 0.05 inch greater in diameter than those from the small seed and 0.6 inch taller and 0.02 inch greater in diameter than those from the ungraded seed.

By reference to Figure 1 it is apparent that stalks from the small seed showed no tendency to catch up with those from the large seed until after the large ones had almost matured their ears; consequently the ears from the small grade were later in maturing. This fact is emphasized by the data presented in Table III, which shows that when the corn from the large grade of seed was in full tassel only 20 per cent from the small grade and 50 per cent from the ungraded seed had reached the tassel stage. Also, when the corn from the large seed was in full silk that from the small grade was only 10 per cent in silk and that from the ungraded seed but 25 per cent. Finally, when the ears on the stalks from large seed were harvested, 95 per cent were in the best canning stage, whereas but 15 per cent from the small seed and 30 per cent from the ungraded seed had reached that stage. These data indicate that the variation in maturity of corn is due to the difference in size of the parent seed.

TABLE III.—*Summary of plant-growth data in sweet corn*

	Grade of seed		
	Large	Small	Ungraded
Average size of stalks 9 days of age:	*Inches*	*Inches*	*Inches*
Height	13.2	9.05	11.15
Diameter	.173	.125	.150
Average size of same stalks at maturity:			
Height	53.4	51.8	52.7
Diameter	.75	.70	.73
Percentage of stalks in full tassel when large grade was in full tassel	100	20	50
Percentage of stalks in full silk when large grade was in full silk	100	10	25
Percentage of corn mature when large grade was gathered	95	15	30

In yield per plant the stalks from large seed gave slightly more than those from small ones, which was occasioned by the fact that two yielded more than one ear. The size of the ears in each type was about the same. In the field test at Shelbyville the stalks from the large kernels yielded 1.3 times as much as those from the small ones.

There was no difference in the number of tillers between the two types. The average number for the large seed was 2.13 and for the small seed, 2.15.

The ears were examined to determine whether the size of the parent kernel influences the size of the kernel in the offspring, and it was found that the sizes varied in both types throughout the same range, the average being about the same. It was therefore concluded that the progeny of large kernels is just as desirable for packing as that from small ones. The Shelbyville test also demonstrated this fact, hence there is no objection to grading seed from this standpoint.

DISCUSSION

Throughout all the various phases of these investigations the economic importance of grading sweet-corn seed for canning purposes was kept in mind. Each experiment demonstrates the difference in rate of growth and time of maturity between the plants from large and small kernels. The stalks from large seed have in all cases been more rapid in growth and more even in maturity than those from small seed. They have also been more resistant to adverse weather conditions and have produced larger yields. For the sake of greater uniformity in date of maturity, it would seem wise to plant the large and small kernels in separate parts of the field, so that the grain may be harvested when it has reached the proper stage without the necessity of picking the whole field twice.

SUMMARY

The large and small kernels of sweet corn germinate and the seedlings come up at about the same time. There is a slight variation in each type, but one has no distinct advantage over the other.

The seedlings from the large kernels are nearly always larger than those from the small ones, and usually remain so. This difference is apparent from the earliest stages.

The large seedlings grow more rapidly and become established in the soil more quickly than the small ones. Consequently, they pass from one stage of development to another in advance of those from the small kernels.

Plants from large kernels reach the tasseling, pollen-shedding, full-silk, and canning stages about five days before those from small kernels. Hence, large seed within a variety tends to produce early maturity and greater uniformity.

Sweet corn for canning purposes should be graded. The large and small kernels should be planted separately.

No difference was found in the size of kernels produced on the ears from large seed and those from small.

There was a larger number of two-eared stalks among those from the large kernels than from the small ones.

More barren and unproductive stalks were found in the plot planted with small kernels than in that planted with large ones.

LITERATURE CITED

(1) Clark, V. A.
1904. seed selection according to specific gravity. N. Y. State Agr. Exp. Sta. Bul. 256: 367–425, illus.

(2) Degrully, L.
1898. sélection des blés et autres semences par la densité. Prog. Agr. et Vit. 30: 453–455.

(3) Dehérain, P.—P., and Dupont, C.
1902. culture du blé au champ d'expériences de grignon, en 1902. Compt. Rend. Acad. Sci. [Paris] 135: 654–657.

(4) Haberlandt, F.
1866. ueber den einfluss des samens auf den ernteertrag. Böhmisches Centbl. Gesam. Landeskultur 1866: 4. [Original not seen. Abstract in Jahresber. Agr. Chem. (1866) 9: 298–300. 1868.]

(5) JOHANNSEN, W. L.
1913. ELEMENTE DER EXAKTEN ERBLICHKEITSLEHRE. Aufl. 2, 724 p., illus. Jena.
(6) KIESSELBACH, T. A., and HELM, C. A.
1917. RELATION OF SIZE OF SEED AND SPROUT VALUE TO THE YIELD OF SMALL GRAIN CROPS. Nebr. Agr. Exp. Sta. Research Bul. 11, 73 p., illus.
(7) ——— and COOK, G. C.
1924. THE RELATIVE EFFECTS OF FOREIGN POLLEN UPON THE KERNEL WEIGHT OF COMMERCIAL VARIETIES AND SELFED STRAINS OF CORN. Jour. Amer. Soc. Agron. 16: 30–36.
(8) LEIGHTY, C. E.
1912. CORRELATION OF CHARACTERS IN OATS, WITH SPECIAL REFERENCE TO BREEDING. Ann. Rpt. Amer. Breeders' Assoc. (1911) 7: 50–61.
(9) LOVE, H. H.
1912. A STUDY OF THE LARGE AND SMALL GRAIN QUESTION. Ann. Rpt. Amer. Breeders' Assoc. (1911) 7: 109–118.
(10) MYERS, C. H.
1912. EFFECT OF FERTILITY UPON VARIATION AND CORRELATION IN WHEAT. Ann. Rpt. Amer. Breeders' Assoc. (1911) 7: 61–74, illus.
(11) PEARL, R., and SURFACE, F. M.
1915. GROWTH AND VARIATION IN MAIZE. Ztschr. Induktive Abstam. u. Vererbungslehre 14: 97–203, illus.
(12) REED, H. S.
1919. GROWTH AND VARIABILITY IN HELIANTHUS. Amer. Jour. Bot. 6: 251–271, illus.
(13) RENICH, M. E.
1921. GROWTH AS RELATED TO SPECIFIC GRAVITY AND SIZE OF SEED. Trans. Ill. Acad. Sci. 14: 109–139, illus.
(14) SANBORN, J. W.
1893. SELECTION OF SEED. Utah Agr. Exp. Sta. Ann. Rpt. (1892) 3: 133–137.
(15) VRIES, H. DE.
1909. THE MUTATION THEORY. Translated by J. B. Farmer and A. D. Darbishire. v. 1, illus. Chicago.
(16) WOLLNY, E.
1877. UNTERSUCHUNGEN ÜBER DIE WERTHBESTIMMUNG DER SAMEN ALS SAAT- UND HANDELSWAARE. Jour. Landw. 25: 75–116, 133–169.

THE MAINTENANCE REQUIREMENT OF DRY COWS [1]

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INTRODUCTION

The determination of the maintenance requirements of farm animals is one of the most important of the basic problems of animal industry. The maintenance requirement is the nutriment necessary for sustenance alone, under the living conditions of production. It is therefore the basic requirement to which must be added the direct cost in nutriment of the material product or the labor sought. It is a permanent "overhead" the extent of which must be known before total requirements and costs of production can be satisfactorily determined.

Several methods have been used in the determination of maintenance requirements. Two of these, at least, are of such nature as to yield data of scientific significance.

With man and with carnivora the determination of the body losses during complete fast have yielded relatively accurate measures of the nutriment required to repair tissue waste and to supply the energy needed to keep the body machinery running. With herbivora the problem becomes much more complex, because with these animals the capacity and the anatomy of the digestive tract are such as to render difficult and uncertain the attainment of complete and actual post-resorptive fast.

Fortunately there is another angle of approach to this problem, namely, that proposed by Rubner in his work on isodynamic replacement, and amplified by Armsby (*2*)[3] for the purpose of determining maintenance requirements. This method compares the effects produced by different amounts of feed, such effects being considered as constituting a linear function of the amount of the feed. The essential correctness of this postulate, within certain limits, has been established by the classic experiments of both Rubner and Armsby.

As an illustration of the method the writers quote from Armsby (*2, p. 34*):

> The addition of 2.1 kilograms of timothy hay, equivalent to 3.575 therms of metabolizable energy, to the basal ration reduced the loss of energy from the

[1] Received for publication Apr. 30, 1925; issued January, 1926.

[2] The experiments on which this paper is based were planned by H. P. Armsby. Responsibility for execution of the details rested largely with J. A. Fries. The computations were made, in large part, by W. W. Braman. The writers wish to express their appreciation of the assistance of E. B. Forbes, director of the institute, in the preparation of the data. They are especially indebted to Max Kriss for his assistance in making the intricate computations and handling the animals during the experiments. They also acknowledge the efficient work of C. D. Jeffries, W. J. Sweeney, R. M. Meredith, Raymond Peterson, and others, who at various times gave their services for the success of the involved and exacting experiments.

[3] Reference is made by number (italic) to "Literature cited," p. 1081.

body of the animal by 2.020 therms. [The basal ration supplied 5.687 therms of metabolizable energy and the losses of body protein and fat were equivalent to 2.377 therms per day.] Evidently, then, to have reduced it by 2.377 therms, that is, to zero, would have required the addition of $2.1 \times \frac{2.377}{2.020} = 2.471$ kg. of the hay, equivalent to 4.207 therms of metabolizable energy. The total maintenance ration of this particular feeding stuff, then, would have been the basal ration plus this amount, or 5,670 kg. of the hay, equivalent to 9.894 therms of metabolizable energy.

It will be noted that the above data are expressed in terms of metabolizable energy. So far as the method is concerned it is immaterial whether results are expressed in terms of metabolizable or of net energy but, for reasons which are set forth more fully in the discussion to follow, net-energy values have been used throughout this study.

As to the variability of the maintenance requirements of farm animals, it was expected that one species would differ from another, and that one individual would differ from another of the same species, and the writers found it so. Size, weight, temperament, variations of body temperature as between individuals and from day to day in the same individual, digestive activity, sex, physical condition, age, variation in the time spent lying as compared with the time spent standing—all these, theoretically at least, have a definite bearing on the maintenance requirement. External conditions, such as thermal environment, seasonal variation (*10, p. 33*), the presence of attendants, and annoyance caused by insects, also have bearing, not only on the maintenance requirement, but also on the accuracy of the determination of it.

A consideration of these many factors which contribute to the maintenance requirement indicates the desirability of directly determining this requirement of an experimental subject, in research on problems of production, rather than applying average figures for maintenance.

In the experiments of this series, the general plan was to determine the maintenance requirement of each individual and to apply these data in the determination of the net-energy values of feeds for milk production and for body increase with this same animal. In a number of instances the writers were unable to carry out this plan and were compelled to make use of average figures.

A perusal of the details of the experiment shows that the heat production and the gaseous excretion were determined on only two days, the ninth and tenth, of the experimental periods. It has been assumed that these two days are representative of the period. That this assumption may introduce an error into the values is undeniable, but that it is not seriously disqualifying is shown by the uniformity of similar data recorded in the work of Armsby and Fries (*1*), and by the agreement of results from the one experimental day with those from the other.

It will also be noted that these present experiments represent the 24-hour metabolism with normal activity as to standing and lying, and therefore should not be considered as representative of complete repose.

The procedure and the general plan of experimentation have been fully outlined in a previous paper (*4*).

PLAN OF THE INVESTIGATION

The general routine of these experiments with cows was the same as in the previous work with steers. The experimental ration was fed during a definite period of not less than three weeks. This comprised a preliminary feeding period of about 11 days, and an experimental or "digestion" period of 10 days during which the visible excreta were collected. During the ninth and tenth days of the digestion period the animal was kept in the respiration calorimeter to permit of measurement of the water vapor, carbon dioxide, methane, and heat emission.

PROGRAM OF EXPERIMENTS

Attention is called to the details of Table I. Four different animals were used, the experiments extending over an interval of three years. The four cows used were Nos. 874, 885, 886, and 887.

TABLE I.—*Periods and rations*

Experiment No.	Cow No.	Period	Preliminary feeding	Calorimeter days	Excreta collected	Daily rations		Feed refused (average per day)
						Grain	Alfalfa hay	
						Kg.	*Kg.*	*Kg.*
221D–1919–20	885	I	Dec. 2–Dec. 30	Jan. 7–Jan. 8	Dec. 30–Jan. 9	2.668	1.779	0.252
	885	II	Jan. 15–Jan. 27	Feb. 4–Feb. 5	Jan. 27–Feb. 6	4.200	2.800	0.728
	885	III	Feb. 8–Mar. 2	Mar. 10–Mar. 11	Mar. 2–Mar. 12	2.668	1.779	0.009
	886	I	Dec. 2–Dec. 9	Dec. 16–Dec. 17	Dec. 9–Dec. 19	2.614	1.745	None.
	886	II	Dec. 25–Jan. 13	Jan. 21–Jan. 22	Jan. 13–Jan. 23	4.200	2.800	0.020
	886	III	Jan. 25–Feb. 17	Feb. 25–Feb. 26	Feb. 17–Feb. 27	2.614	1.745	None.
221E–1921	885	I	Jan. 22–Feb. 1	Feb. 9–Feb. 10	Feb. 2–Feb. 11	3.760	2.540	0.946
	885	II	Feb. 12–Feb. 22	Mar. 2–Mar. 3	Feb. 23–Mar. 4	2.360	1.580	0.009
221F–1921–22	874	I	Dec. 3–Dec. 13	Dec. 21–Dec. 22	Dec. 14–Dec. 23	4.100	2.733	None.
	874	II	Jan. 7–Jan. 17	Jan. 25–Jan. 26	Jan. 18–Jan. 27	2.744	1.830	None.
	887	I	Feb. 4–Feb. 14	Feb. 22–Feb. 23	Feb. 15–Feb. 24	3.696	2.464	None.
	887	II	Feb. 25–Mar. 7	Mar. 15–Mar. 16	Mar. 8–Mar. 17	2.474	1.650	None.

Cow 885 was dry during two successive years, having failed to get with calf during the second year, and was used twice in connection with the maintenance experiments. Cows 874, 886, and 887 were used during only one year in this study of maintenance requirements. All these animals were used in experiments on milk production, the results of which will be reported in a separate paper.

ANIMALS

The subjects used were either purebred or grade Jerseys of average size and quiet disposition.

Cow 885 was a purebred Jersey, registry No. 333236, dropped March 27, 1914. She gave birth to her first calf September 15, 1917, and was not bred again prior to the beginning of this study.

Cow 886 was a purebred Jersey, registry No. 333886, dropped July 13, 1914. She gave birth to her first calf October 10, 1917, and was bred again just prior to the beginning of this study.

Cow 874, of unknown breeding, was dropped May 5, 1916. She was typically Jersey in conformation and color, was fresh twice, the

second time on July 27, 1920, and was not bred again prior to the beginning of this study.

Cow 887, a purebred Jersey, was dropped September 5, 1917. She was fresh twice, January 11, 1920, and March 11, 1921, and was not bred again prior to the beginning of this study.

RATIONS

The same feeding stuffs, mixed in the same proportions, were fed throughout the experiment. The quantity fed each animal was so adjusted as to maintain approximately constant live weight in experiments 221D–885–I and III, experiments 221D–886–I and III, experiment 221E–885–II, experiment 221F–874–II, and experiment 221F–887–II. In the other periods, 221D–885–II, 221D–886–II, 221E–885–I, 221F–874–I, and 221F–887–I the feed was increased so that the animals gained materially in body weight.

The grain mixture fed was composed of 30 parts wheat bran, 30 parts ground oats, 30 parts corn meal, and 10 parts old process linseed meal, all of good quality. The roughage was good-quality alfalfa, grown in Colorado, nicely cured, of uniform green color, containing a normal proportion of leaves.

The details of methods of handling, weighing, sampling and analysis of feeds, feces and urine have been published (*4, p. 8–9*).

For a description of the respiration calorimeter and the minutiae of methods for its operation, reference is made to the publications of Armsby and Fries.

DIGESTIBILITY OF THE RATION

Owing to the impossibility of obtaining attendants, it was impracticable to collect the dung and urine separately during the digestion period. Hence, in order to get an approximate value for the apparent digestibility of the ration, the dung and urine were collected separately on one day of the experimental period by members of the institute staff. On the other days of the digestion period the dung and urine were collected together, by means of a conducting apron devised by one of the writers, Fries.

Assuming the relative constancy of composition of the dry matter of the dung under the conditions of the experiment, it is possible, by making use of the percentage of crude fiber in the dung and urine mixture, and in the dung alone, to approximate the apparent digestibility of the various constituents of the feed. The details of this computation will be more fully discussed later.

LIVE WEIGHT

The animals were weighed daily after the morning feeding and before watering. During the 48 hours spent in the respiration calorimeter live weights could not be taken. The animals, however, were weighed on entering the chamber and again on leaving it. For the average weight of the animals during the experimental periods the weights on the 10 days immediately preceding the calorimeter days, including the last two days of the preliminary period, have been used (Table II).

TABLE II.—*Average daily live weights of experimental subjects*

Experiment and cow Nos.	Period I	Period II	Period III
	Kg.	*Kg.*	*Kg.*
221D-885	415.22	429.74	[a] 426.36
221D-886	399.78	424.41	420.19
221E-885	443.12	434.36	.....
221F-874	428.80	415.58	.....
221F-887	335.21	320.49	.....

[a] Only 9 days in Period III, 221D-885.

DETAILS OF ANALYSES

The customary feeding-stuff analyses were made on the alfalfa hay, grain mixture, and excreta. These data, together with the heat of combustion, have been computed to a dry-matter basis, and are presented in Tables III, IV, and V.

TABLE III.—*Composition of the dry matter of the feed*

Substance	Experiment No.	Cow and period No.	Ash	Protein [a]	Nonprotein [b]	Crude fiber	Nitrogen-free extract	Ether extract	Total nitrogen	Protein nitrogen	Carbon	Energy per gram
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*
Alfalfa hay	221D	885-I	7.85	11.09	2.36	37.86	36.80	2.03	2.28	1.77	46.08	4.53943
		886-I										
		885-II	8.38	12.11	2.42	34.18	40.84	2.07	2.45	1.94	46.12	4.52408
		886-II										
		885-III										
		886-III	8.44	11.61	2.49	34.75	40.75	1.97	2.39	1.86	46.05	4.57295
	221E	885-I-II	7.84	12.33	2.47	33.02	42.40	2.00	2.49	1.97	46.06	4.49728
	221F	874-I-II	8.03	11.52	2.18	36.93	39.70	1.64	2.31	1.84	46.55	4.54493
		887-I-II	8.13	11.54	2.19	34.47	41.97	1.71	2.31	1.85	46.12	4.51322
Grain mixture	221D	885-I	4.69	13.13	2.14	9.02	66.12	4.90	2.70	2.24	46.25	4.62553
		886-I										
		885-II	4.57	12.79	2.21	10.62	65.05	4.76	2.65	2.18	46.26	4.62276
		886-II										
		885-III										
		886-III	4.56	12.66	2.17	9.54	66.45	4.62	2.62	2.16	46.09	4.66089
	221E	885-I-II	4.44	14.45	0.68	9.09	66.77	4.57	2.61	2.47	46.55	4.60654
	221F	874-I-II	3.99	12.48	1.83	9.15	67.67	4.88	2.52	2.13	46.56	4.65669
		887-I-II	4.15	12.61	1.88	9.15	67.46	4.76	2.55	2.15	46.49	4.66785
Refused feed	221D	885-I	6.43	14.69	2.80	10.82	61.28	3.99	2.95	2.35	45.61	4.54953
		885-II	6.55	14.78	2.59	10.42	63.19	2.46	2.92	2.37	45.91	4.58022
	221E	885-I	7.71	13.89	2.07	11.60	60.76	3.96	2.81	2.37	44.96	4.47602

[a] True protein; A. O. A. C. method. [b] Nonprotein, N×4.7.

TABLE IV.—*Composition of the dry matter of the dung alone*

Experiment, animal, and period Nos.	Ash	Protein [a]	Nonprotein [b]	Crude fiber	Nitrogen-free extract	Ether extract	Total nitrogen		Carbon		Energy per gram
							From fresh substance [c]	From air-dry substance	From fresh substance	From air-dry substance	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*
221D–885–I	11.88	10.23	1.95	37.52	35.95	2.48	2.05	2.00	[d]	46.67	4.66491
II	12.40	11.53	2.15	36.34	35.30	2.29	2.30	2.09	[e]	46.69	4.68633
III	12.27	10.61	2.05	37.71	34.96	2.39	2.13	2.07	[e]	46.59	4.68649
221D–886–I	12.42	9.72	1.98	38.48	34.78	2.63	1.98	1.89	[e]	46.51	4.62183
II	12.89	10.71	2.54	35.05	36.26	2.55	2.25	2.11	[e]	46.10	4.56661
III	12.41	11.28	2.03	36.93	34.86	2.51	2.24	2.22	[e]	46.28	4.63401
221E–885–I	11.26	10.12	1.50	38.04	36.94	2.15	1.94	1.80	[e]	46.84	4.69723
II	11.78	11.56	0.91	37.11	36.58	2.06	2.04	1.97	[e]	46.28	4.63002
221F–874–I	11.28	10.86	1.66	34.79	39.03	2.37	2.09	2.00	48.00	46.95	4.70966
II	12.19	10.16	1.69	35.42	38.16	2.39	1.93	1.99	47.23	46.02	4.69322
221F–887–I	11.33	10.24	3.29	31.90	40.99	2.25	2.34	1.97	49.20	45.82	4.69662
II	11.06	10.21	2.51	38.67	35.40	2.16	2.17	1.93	48.05	46.16	4.68011

[a] True protein; A. O. A. C. method. [b] Nonprotein, N×4.7. [c] By König's method.
[d] No determinations of carbon in the fresh substance were made prior to experiment 221F.
[e] By direct combustion of the fresh material.

TABLE V.—*Composition of the dry matter of the dung and urine mixture*

Experiment, animal, and period Nos.	Ash	Protein [a]	Nonprotein [b]	Crude fiber	Nitrogen-free extract	Ether extract	Total nitrogen		Carbon		Energy per gram
							From fresh substance [c]	From air-dry substance	From fresh substance	From air-dry substance	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories*
221D–885–I	18.46	8.98	26.28	30.46	13.41	2.42	7.25	3.21	[d]	43.24	4.39446
II	17.24	9.36	23.30	29.97	17.64	2.49	6.66	2.88	[d]	43.72	4.41033
III	19.22	9.21	24.28	28.25	16.30	2.74	6.85	3.00	[d]	42.89	4.33180
221D–886–I	17.43	8.84	22.38	29.81	18.54	3.00	6.38	2.81	[d]	43.22	4.34790
II	17.89	9.46	24.32	28.81	16.95	2.58	6.86	3.10	[d]	43.17	4.34358
III	17.83	9.81	22.80	29.58	17.51	2.48	6.60	2.63	[d]	43.58	4.35050
221E–885–I	17.03	9.98	13.33	29.07	27.98	2.61	5.97	2.78	[d]	44.01	4.42019
II	18.11	9.63	24.15	29.15	16.72	2.25	6.89	2.93	46.58	43.67	4.32726
221F–874–I	15.30	9.02	23.12	29.80	20.44	2.32	6.36	2.85	47.03	43.80	4.42538
II	16.48	8.64	23.10	28.53	20.72	2.52	6.30	2.98	47.53	43.85	4.38052
221F–887–I	16.85	8.82	25.05	29.85	17.33	2.11	6.74	2.40	47.95	44.21	4.45052
II	17.26	9.09	26.79	28.19	16.57	2.10	7.15	2.59	47.63	43.89	4.39412

[a] True protein, A. O. A. C. method.
[b] Nonprotein, N×4.7.
[c] No determinations of carbon in the fresh substance were made prior to experiment 221E.
[d] By direct combustion of the fresh materials.

The analyses of the dung alone represent the composition of the dung as collected separately on a single day of each period, and are used only to obtain an approximation of the apparent digestibility. For purposes of comparison, the total nitrogen was determined on the fresh material by König's method, and also on the sample after air drying by the Kjeldahl method. The data show clearly that nitrogen was lost during the drying of the sample. Beginning with experiment 221F the carbon in the fresh material also was determined by direct combustion as well as in the air-dry sample. The loss of carbon shown is of relatively greater consequence in subsequent computations than is the loss of nitrogen, and may not be neglected without introducing material error.

The results obtained by the analysis of the dung and urine mixture as shown in Table V are used to obtain the balance of matter, and are the basis of all computations except those relating to the apparent digestibility. Here again the loss of nitrogen and carbon during drying is evident, this loss apparently being due to fermentation.

THE APPARENT DIGESTIBILITY OF THE RATION

In order that the method by which the writers have arrived at an approximation of the apparent digestibility of the various constituents of the ration may be clear, the computation for experiment 221D–885–I has been included in detail in Table VI. The method is based primarily on the assumption that the percentage composition of the dry matter of the dung is approximately constant for a given ration, an hypothesis which seems to be justified by an examination of data previously published from the institute. In connection with the second part of the computation (the fresh weight of the dung alone) it has been necessary to assume the percentage dry matter of the dung alone to be a constant on a given ration. There is a measure of uncertainty in this assumption. However, since this factor affects only the urine, no great error results from this procedure.

TABLE VI.—*Example of computation of apparent digestibility (221D–885–I)*

FEEDS AND EXCRETA; FRESH AND DRY BASIS

Average daily feeds and excreta; fresh weights; 10-day period		Dry matter	
	Kg. per day	*Per cent*	*Grams per day*
Alfalfa hay	1.779	89.26	1,588
Mixed grain	2.668	86.28	2,302
Salt	.030	98.77	30
Refused feed	.252	88.25	223
Dung and urine	9.415	14.29	1,346
Apron scrapings	.020	14.29	3
Computed dung alone	4.574		1,095
Computed urine alone	4.862		254
Spilled urine	.007		
Total urine	4.869		254
Totals:			
Dung and urine mixture	9.443		
Dung alone	4.574		
Urine alone	4.869		

CONSTITUENTS OF FEEDS AND EXCRETA

	Dry matter	Organic matter	Protein [a]	Nonprotein [b]	Crude fiber	Nitrogen-free extract	Ether extract	Total nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*
Salt	29.6									
Alfalfa hay	1,588.0	1,463.3	176.2	37.5	601.1	616.2	32.2	36.2	731.7	7,208.6
Mixed grain	2,302.0	2,194.0	302.2	49.3	207.7	1,522.1	112.8	62.1	1,064.6	10,648.0
Refused feed	222.6	208.3	32.7	6.2	24.1	136.4	8.9	6.6	101.5	1,012.7
Total eaten	3,697.0	3,449.0	445.7	80.6	784.7	2,001.9	136.1	91.7	1,694.8	16,843.9
Dung	1,095.0	964.9	112.0	21.4	410.8	393.6	27.2	22.5	500.5	[c] 4,949.0
Total digested	2,602.0	2,484.1	333.7	59.2	373.9	1,608.3	108.9	69.2	1,194.3	11,894.9
Per cent digested	70	72	75	73	48	80	80	75	70	71

[a] True protein; A. O. A. C. method.

[b] Nonprotein, N×4.7.

[c] Corrected for loss on drying (see Table VIII for method and factors used):

Computation of dung in mixture:

Dry matter of dung and urine mixture : X :: per cent crude fiber in dung alone : per cent crude fiber in dung and urine mixture.

$$\frac{1,349\times 30.46}{37.52}=1,095.0 \text{ grams.}$$

Computation of fresh weight of dung:

Dry matter of dung alone (computed) : X :: per cent of dry matter of dung alone : 100

$$\frac{1,095.0}{23.94}\times 100=4,574 \text{ grams.}$$

In Table VII the coefficients of apparent digestibility, computed as explained above, have been collected. The averages are arithmetic, not weighted, means.

TABLE VII.—*Coefficients of apparent digestibility (feed minus feces)*

Experiment, animal, and period Nos.	Dry matter	Organic matter	Protein	Nonprotein •	Crude fiber	Nirtogen free-extract	Ether extract	Total nitrogen	Carbon	Organic matter as energy
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
221D-885-I	70	72	75	73	48	80	80	75	70	71
II	70	72	72	72	49	81	82	73	69	69
III	74	75	77	77	50	84	82	78	73	73
Average	71	73	75	74	49	82	81	75	71	71
221D-886-I	72	74	78	75	48	82	80	78	71	71
II	70	72	75	67	48	80	79	74	70	70
III	72	73	74	75	47	82	80	75	71	71
Average	71	73	76	72	48	81	80	76	71	71
221E-885-I	73	74	80	68	49	82	83	79	72	72
II	73	74	76	82	45	82	84	78	72	73
Average	73	74	78	75	47	82	84	79	72	72
221F-874-I	68	70	71	73	46	78	79	73	67	67
II	70	72	74	74	47	79	80	75	69	69
Average	69	71	73	74	47	79	80	74	68	68
221F-887-I	68	69	73	46	46	77	79	69	65	67
II	74	75	78	67	48	84	84	77	73	74
Average	71	72	76	57	47	81	82	73	69	71

• Nonprotein, N×4.7.

THE LOSS OF NITROGEN AND CARBON ON DRYING SAMPLES OF EXCRETA PRIOR TO GRINDING

The loss of nitrogen and carbon in drying during the preparation of the samples for analysis has been a recognized source of error throughout the entire series of investigations with the respiration calorimeter. In the experiments with steers the feces and the urine were handled separately, and the loss on drying the feces was found to be small, and was disregarded; while the energy was corrected for the urinary loss on the basis of Rubner's figure of 5.45 Cal. per gram of nitrogen lost on drying. The data for the first experiment with cows, 221A, were also handled in this way, as the feces and urine were collected separately.

In all experiments with these cows subsequent to 221A it was necessary to collect the feces and urine as a mixture, and it was soon recognized that the physical state of this mixture, particularly its moisture content, might be important in relation to fermentation during preparation for analysis. The decomposition of these mixed samples during drying was greater than that of either the dung or the urine alone, and especially significant was it that more carbon was lost than could be accounted for as originating in the nitrogenous compounds of the urine in the mixture. There is a possibility, however, that the loss of nitrogen observed may be somewhat less than the true loss, since fixation of nitrogen has been shown to occur in drying feces (*8*).

This loss of carbon unaccompanied by nitrogen may result from two sources, viz, from a continuation of the intestinal fermentation after the feces have been voided, and from the breaking up of the nonnitrogenous compounds in the urine. That both processes contribute to this excess of carbon dioxide seems probable. However, the present state of the writers' knowledge of the compounds involved in the decomposition of the urine is insufficient to serve as the basis for the correction of its energy, and this has not been attempted. Neither has the energy of the urine been corrected for the free ammonia and free carbon dioxide present.

When the sources of the carbon dioxide lost during the drying of the mixed feces and urine, in excess of that coming from the nitrogenous compounds of the urine, are considered, to wit, the crude fiber and the nitrogen-free extract of the dung, the writers are impressed with the possibility of extensive error. Soon after the mixture is placed in the drying closet a thick scum forms over the surface, this scum preventing rapid drying and at the same time maintaining in the interior of the mass conditions favorable for the continuance of digestive cleavage and for the development of bacteria with the resultant formation of methane and carbon dioxide. This condition was not recognized early enough in this series of experiments to have permitted of direct determinations of the amounts of the gases lost. However, in all experiments following 221E the total carbon was determined by direct combustion of the fresh material, and this, in conjunction with the total nitrogen as determined in the fresh substance by König's method, furnishes sufficient data to make possible a computation of the energy loss due to the aforementioned factors.

It is apparent, then, that the carbon lost on drying the mixed feces and urine must be divided into three parts, in so far as its relation to the corresponding energy loss is concerned: (1) That portion which is combined with nitrogen and is accounted for through the use of Rubner's factor of 5.45 Cal. per gram of nitrogen lost on drying; (2) that portion which is present in solution as part of the system $NH_3.CO_2.H_2O$ (this is to some extent compensated for by the fact that a portion of the NH_3 present in this system has been included in the total nitrogen lost on drying, its carbon equivalent thus being taken into account, as will later appear); (3) the residuum of carbon considered as originating in the fermentation of the nonnitrogenous material.

Since this fermentation simulates intestinal fermentation, not only carbon dioxide but also methane is produced. This latter the writers have been unable to determine quantitatively. However, in view of the relatively small difference between the heats of combustion of crude fiber and nitrogen-free extract, the writers feel justified in computing the amount of energy lost through this external fermentation from the carbon dioxide produced and the energy equivalent of starch, as representing nitrogen-free extract generally.

Since in experiment 221D the carbon was not determined in the fresh dung and urine mixture, it was necessary to compute the correction for this experiment on the basis of average figures. The data upon which this computation is based are indicated in Table VIII.

TABLE VIII.—*Carbon and nitrogen lost per day through the drying of the dung and urine mixture*

Experiment, animal, and period Nos.	Dry matter [a]	Water [a]	Nitrogen lost	Carbon lost			Energy equivalents		Energy of air-dry material	Energy of air-dry material, corrected—	
				Total	Combined with nitrogen	From carbohydrate	N × 5.45	Starch C × 9.4		For nitrogen	For nitrogen and carbohydrate fermentation
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221D–885–I	1,349.1	8,093.5	[b] 54.5	[c] 62.7	23.4	[c] 39.3	297.0	[c] 369.4	5,928.6	6,225.6	6,595.0
II	1,982.9	14,682.9	[b] 74.8	[c] 86.0	32.1	[c] 53.9	407.7	[c] 506.7	8,745.7	9,153.4	9,660.1
III	1,355.0	9,097.9	[b] 52.3	[c] 60.1	22.4	[c] 37.7	285.0	[c] 354.4	5,869.3	6,154.3	6,508.7
221D–886–I	1,396.4	7,901.2	[b] 49.9	[c] 57.4	21.4	[c] 36.0	272.0	[c] 338.4	6,071.4	6,343.4	6,681.8
II	2,200.3	13,434.2	[b] 82.8	[c] 95.2	35.5	[c] 59.7	451.3	[c] 561.2	9,557.2	10,008.5	10,569.7
III	1,347.8	9,161.5	[b] 53.5	[c] 61.5	23.0	[c] 38.5	291.6	[c] 361.9	5,863.6	6,155.2	6,517.1
221E–885–I	1,672.8	10,180.5	[b] 53.2	[c] 61.2	22.8	[c] 38.4	289.9	[c] 361.0	7,394.1	7,684.0	8,045.0
II	1,218.3	7,799.5	48.1	69.3	20.6	48.7	262.1	457.8	5,271.9	5,534.0	5,991.8
221F–874–I	2,225.8	11,144.2	78.3	71.9	33.6	38.3	426.7	360.0	9,850.0	10,276.7	10,636.7
II	1,516.0	7,512.4	50.3	58.8	21.6	37.2	274.1	349.7	6,641.5	6,915.6	7,265.3
221F–887–I	1,887.4	11,543.3	82.0	70.7	35.2	35.5	446.9	333.7	8,400.1	8,847.0	9,180.7
II	1,290.5	7,626.2	59.2	48.5	25.4	23.1	322.6	217.1	5,697.0	6,019.6	6,236.7
Cows in milk											
221E–886–I			[b] 79.9	[c] 91.9	34.3	[c] 57.6	435.5	[c] 541.4			
II			75.9	69.8	32.6	37.2	413.7	349.7			
221E–874–I			[b] 65.2	[c] 75.0	28.0	[c] 47.0	355.3	441.8			
II			[b] 60.0	[c] 69.0	25.7	[c] 43.3	327.0	[c] 407.0			
221F–886–I			83.9	119.6	36.0	83.6	457.3	785.8			
II			96.8	124.7	41.5	83.2	527.6	782.1			
221G–887–I			75.7	108.3	32.5	75.8	412.6	712.5			
II			58.5	82.2	25.1	57.1	318.9	536.7			
III			61.4	79.3	26.3	53.0	334.6	498.2			
IV			87.3	83.5	37.5	46.0	475.8	432.4			

[a] Not corrected for loss on drying.
[b] Not included in average used to obtain data for carbon correction.
[c] Computed factors:
1 gram C ⇔ 2.25 grams starch.
1 gram N lost ⇔ 1.15 grams carbon lost.
1 gram carbon lost ⇔ 0.627 gram carbon from fermentation of carbohydrates.
1 gram N ⇔ 3.43 grams ammonium carbonate.
1 gram carbon of carbohydrate fermented (starch) ⇔ 9.4. Cal.
Ratio of carbon to nitrogen in urea or in ammonium carbonate $\frac{C}{N}$ = 0.429.

In Table IX similar data for the loss during the drying of the dung alone have been compiled for reference. The dung alone is used only in arriving at approximate figures for the apparent digestibility, and the data available for the loss on drying are few and widely variable. Therefore, it has seemed best not to apply the correction to the carbon and energy of the dung alone in this particular series of experiments, but rather to place the data on record in order to call attention to the fact that the loss of carbon, and its equivalent energy, during the preparation of samples of feces for analysis, is a material and a variable factor which must be taken into account in exact experimentation involving digestibility.

TABLE IX.—*Carbon and nitrogen lost per day during the drying of dung alone*

Experiment, animal, and period Nos.	Dry matter [a]	Water	Nitrogen lost	Carbon lost			Energy equivalents		Energy of air-dry material	Energy of air-dry material, corrected—	
				Total	Combined with nitrogen. N× 0.429	From carbohydrate	N× 5.45	Starch C× 9.4		For nitrogen	For nitrogen and carbohydrate fermentation
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221D–885–I	1,095.0	3,478.7	0.6	[b]	0.3	[c] 9.8	3.3	92.1	5,108.1	5,111.4	5,203.5
II	1,635.7	8,346.0	3.4	[b]	1.5	[c] 48.8	18.5	458.7	7,665.4	7,683.9	8,142.6
III	1,014.2	4,103.1	.6	[b]	.3	[c] 9.8	3.3	92.1	4,753.0	4,756.3	4,848.4
221D–886–I	1,081.7	4,035.1	1.0	[b]	.4	[c] 13.0	5.5	122.2	4,999.9	5,005.4	5,127.6
II	1,808.5	7,541.1	2.5	[b]	1.1	[c] 35.8	13.6	336.5	8,258.7	8,272.3	8,608.8
III	1,079.7	4,776.8	.1	[b]	.0	[c] .0	.5	.0	5,003.3	-------	-------
221E–885–I	1,279.8	4,840.0	1.8	[b]	.8	[c] 26.0	9.8	244.4	6,011.5	6,021.3	6,265.7
II	955.9	3,974.6	.7	[b]	.3	[c] 9.8	3.8	92.1	4,425.8	4,429.6	4,521.7
221F–874–I	1,906.6	7,201.9	1.8	[c] 19.9	[c] .8	[c] 19.1	9.8	179.5	8,979.4	8,989.2	9,168.7
II	1,221.7	3,848.0	None.	[c] 14.7	.0	[c] 14.7	.0	138.2	5,733.7	-------	5,871.9
221F–887–I	1,766.1	7,153.5	6.5	[c] 59.6	2.8	[c] 56.8	35.4	533.9	8,294.7	8,330.1	8,864.0
II	945.4	3,441.0	2.3	[c] 18.0	1.0	[c] 17.0	12.5	159.8	4,424.6	4,437.1	4,596.9
Cows in milk											
221E–886–I	-------	-------	1.3	[b]	.6	[c] 16.3	2.8	-------	-------	-------	-------
II	-------	-------	2.2	[b]	.9	[c] 29.3	4.9	-------	-------	-------	-------
221E–874–I	-------	-------	2.3	[b]	1.0	[c] 29.3	4.9	-------	-------	-------	-------
II	-------	-------	1.7	[b]	.7	[c] 22.8	3.8	-------	-------	-------	-------
221F–886–I	-------	-------	6.6	52.7	2.6	50.1	14.2	-------	-------	-------	-------
II	-------	-------	9.9	75.6	4.0	71.6	21.8	-------	-------	-------	-------
221G–887–I	-------	-------	3.4	30.2	1.4	28.9	7.6	-------	-------	-------	-------
II	-------	-------	1.4	13.5	.6	12.9	3.3	-------	-------	-------	-------
III	-------	-------	2.8	22.1	1.1	21.0	6.0	-------	-------	-------	-------
IV	-------	-------	2.3	78.1	.9	77.2	4.9	-------	-------	-------	-------

[a] Not corrected for loss on drying.
[b] Not determined.
[c] Computed; based on average figures.

In this study the uncorrected dry matter must be used as the basis for the computations, because the analyses were made on the dried substance which had sustained the losses above referred to. Therefore, in the case of the dung and urine mixture, the appropriate corrections have been applied to the individual constituents affected.

PRODUCTION OF EPIDERMAL TISSUE

The cows were not clipped, as were the steers in the earlier experiments. The animals were thoroughly brushed, however, before being placed in the respiration calorimeter, and again on leaving it. The material obtained by this second brushing has been taken as representing the growth of hair, epithelium, etc., during the time spent in the calorimeter. Owing to the inadvertent destruction of a sample, the data for experiment 221D–886–I are incomplete. The average for Periods II and III was therefore used. The data for this loss are included in Table X.

TABLE X.—*Loss as hair and scurf per day*

Experiment, animal, and period Nos.	Dry matter	Nitrogen	Carbon	Energy
	Grams	*Grams*	*Grams*	*Calories*
221D–885–I	13.9	1.3	6.1	65.9
II	16.7	1.9	7.5	81.5
III	14.9	1.8	6.5	71.5
221D–886–I	[a] 12.1	[a] 1.2	[a] 5.4	[a] 59.1
II	14.1	1.3	6.2	67.0
III	10.1	1.1	4.7	51.1
221E–885–I	15.2	1.2	6.0	64.2
II	11.0	1.2	4.6	50.0
221F–874–I	11.6	1.1	5.1	56.0
II	11.8	1.1	5.2	57.2
221F–887–I	13.3	1.4	6.2	68.1
II	11.8	1.2	5.0	55.9

[a] Average of Periods II and III, cow 886.

THE GASEOUS EXCRETA

With the animals in the respiration calorimeter the carbon dioxide, methane, and water vapor excreted were determined by methods which have been described in previous publications of the institute. The data so obtained for this series of experiments are presented in Table XI. It will be noted that the ratio of carbon to hydrogen in the combustible gases excreted by these cows was quite variable. This was in part due to the difficulty experienced in the determination of the hydrogen. However, there was on the average an excess of hydrogen to the amount theoretically required to unite with the carbon to form methane. This finding is borne out by subsequent unpublished experiments, but the variability of the results, due to imperfections in the hydrogen estimation, is such as to render impossible definite interpretation of this apparent excess of hydrogen. In experiment 221D–886–I an accident to the apparatus rendered the completion of the two-day period impossible, and the data recorded are for one day only.

TABLE XI.—*Carbon, hydrogen, and water vapor in daily gaseous excreta* [a]

Experiment, animal, and period Nos.	Water vapor	Carbon dioxide	Carbon [b]	Hydrocarbons			Ratio of carbon to hydrogen $\left(\frac{C}{H}\right)$
				Hydrogen	Carbon	Methane [c]	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
221D–885–I	4,866.7	3,360.9	916.5	26.0	70.2	93.7	2.700
II	6,544.4	4,067.6	1,109.2	31.3	101.3	135.4	3.236
III	5,130.5	3,695.8	1,007.8	29.7	88.2	117.8	2.970
221D–886–I [d]	4,213.3	3,440.0	938.1	31.2	81.7	109.2	2.619
II	6,932.0	4,720.7	1,287.3	39.9	115.9	154.9	2.905
III	3,726.8	3,409.0	929.6	29.1	85.1	113.8	2.924
221E–885–I	6,568.9	3,940.0	1,074.4	41.1	91.0	121.6	2.214
II	4,886.3	3,515.5	958.7	31.6	78.3	104.6	2.478
221F–874 I	5,851.7	4,881.6	1,331.2	41.4	118.1	157.8	2.853
II	4,034.8	3,808.8	1,038.7	29.4	86.0	114.9	2.925
221F–887–I	5,309.5	4,549.1	1,240.5	38.1	114.2	152.6	2.997
II	3,256.9	3,521.8	960.4	28.1	80.7	107.9	2.872

[a] Corrected for man entering chamber to make adjustments.
[b] Computed from CO_2, grams $CO_2 \times 0.2727$.
[c] Computed from carbon in hydrocarbons.
[d] One-day experiment.

THE BALANCE OF MATTER

From the data in the preceding tables the balance of matter per day and head has been computed as recorded in Table XII. The balance of matter represents the average for the 10-day period; the balance of water that of the 2-day calorimeter test. In determining the water balance, on the 2 respiration-calorimeter days, the daily portions of the feeds as previously weighed were reweighed shortly before being introduced into the chamber, any difference being considered as representing a gain or loss of water. Also special samples were taken from the excreta voided in the calorimeter for the determination of dry matter. The nitrogen outgo was determined by analysis of the fresh, undried excreta, and the carbon has been corrected for the loss on drying. The organic hydrogen oxidized in the body has been ignored as being of a magnitude of no material importance in the water balance. The factors and the general method involved are those customarily used, and, as in previous experiments, the schematic body is assumed to consist of two variables, protein and fat, and a constant, glycogen.

TABLE XII.—*The gain or loss of water, protein, and fat per day and per head*

Experiment, animal, and period Nos.	Water	Protein	Fat
	Grams	*Grams*	*Grams*
221D–885–I	−4,063.0	−45.0	+103.9
II	−3,458.4	+28.2	+447.3
III	−1,531.5	+16.2	+28.9
221D–886–I	−9,284.7	+36.0	+72.9
II	−6,586.4	+25.2	+446.9
III	−5,597.2	+33.6	+74.1
221E–885–I	−3,616.3	+103.8	+214.1
II	−1,882.5	+19.8	−28.2
221F–887–I	−6,219.7	+25.8	+302.3
II	−2,393.2	−29.4	+42.9
221F–874–I	−3,721.5	+16.8	+358.4
II	−1,502.7	+6.0	+13.2

The animals, without exception, drank materially less water per day while in the respiration calorimeter than during the "digestion" period. This indicates that they were varying from normal to some extent while in the calorimeter. However, the actual influence of this variation may readily be taken into account in the computation of the heat production, of the metabolizable energy, and of net-energy values.

The data indicate a loss of protein, coincident with a gain of fat, in experiments 221D–885–I and 221F–887–II. In experiment 221E–885–II the apparent balances were reversed; protein was gained while fat was lost. The net balances of dry matter in the maintenance periods show that in all cases more feed was given than was required to maintain equilibrium.

In experimental series 221D the third period for each cow was designed to duplicate the first. In the case of cow 885 it is apparent that the utilization of the feed, as measured by the gain or loss of protein and fat, was not the same. This difference may be ascribed in part to the refusal of feed in Period I, which view is substantiated by a comparison with data obtained in the corresponding periods with cow 886 in which the experimental conditions were identical except that there was no refused feed, the data from the two periods.

being in close agreement. That the effect of the refused feed is so marked in this particular instance is no doubt due to the fact that the cow went badly off feed just prior to the respiration-calorimeter test.

Table XIII sets forth a correction to be applied to the dry matter of the excreta on account of irregularity of excretion. This is necessary because the balances of nitrogen and carbon are computed on the basis of the 10-day period, the correction rendering the outgo during the calorimeter days representative of the digestion period as a whole.

TABLE XIII.—*Correction for irregularity of excretion*

Experiment, animal, and period Nos.	Dry matter of the dung and urine mixture (per day)		Correction
	10-day period *	Calorimeter day *	
	Grams	*Grams*	*Grams*
221D–885–I	1,349.1	955.3	+393.8
II	1,982.9	1,185.5	+797.4
III	1,355.0	996.6	+358.4
221D–886–I	1,396.4	1,033.3	+363.1
II	2,200.3	1,814.1	+386.2
III	1,347.8	1,071.6	+276.2
221E–885–I	1,672.8	1,439.5	+233.4
II	1,218.3	1,069.5	+148.8
221F–874–I	2,494.4	2,254.6	+239.8
II	1,688.5	1,434.0	+254.5
221F–887–I	2,168.7	1,934.3	+234.4
II	1,499.6	1,649.6	−150.0

* Not corrected for loss on drying.

The respiration calorimeter is not equipped for weighing the animal. The subject is weighed, however, just before entering, and again as it is removed from the chamber. In every period there was a loss in live weight during the calorimetric measurements. This is largely due to the failure of the cows to drink as much water while in the calorimeter as during the 10-day digestion period.

As in previous work of the institute, the heat emission of the animal was determined by direct measurement on two consecutive days of the experimental period. The methods used and a description of the apparatus employed have been covered in detail in the various publications of Armsby and Fries. The heat emission per day—by radiation and conduction and as latent heat of water vapor—is given in Table XIV.

TABLE XIV.—*Daily emission and production of heat*

Experiment, animal, and period Nos.	Heat emission	Correction for body gain	Heat production
	Cals.	*Cals.*	*Cals.*
221D–885–I	8,462.8	−82.5	8,380.3
II	9,667.7	−60.3	9,607.4
III	8,845.7	−29.0	8,816.7
221D–886–I	8,252.2	−193.6	8,058.6
II	10,603.8	−129.3	10,474.5
III	8,030.0	−115.9	7,914.1
221E–885–I	9,791.2	−69.5	9,721.7
II	8,773.4	−37.9	8,735.5
221F–874–I	11,037.1	−72.9	10,964.2
II	9,106.3	−29.5	9,076.8
221F–887–I	10,451.1	−123.9	10,327.2
II	8,446.7	−51.7	8,395.0

DAILY EMISSION AND PRODUCTION OF HEAT

It is obvious that the heat emission equals the heat production only in case there is neither gain nor loss of matter by the body during the time of measurement. In the experiments herein considered the animals gained or lost varying amounts of fat and protein. Hence, it is necessary to compute the thermal equivalent of these gains or losses in order to arrive at the heat production of the animal. This computation has the effect to bring the body mathematically to its state as at the beginning of the experiment.

In order that this computation may not be confused with the determination of the energy value of the tissue exchange, the data for experiment 221E–885–I are given in detail.

COMPUTATION OF THE CORRECTION FOR THE TEMPERATURE OF THE GAIN BY THE BODY, EXPERIMENT 221E–885–I

Average body temperature, 38.61° C.; calorimeter temperature, 17.86° C.

Protein	+0.1038 kg.	×0.3	×20.75=	+ 0.65 Cal.
Fat	+0.2141 kg.	×0.66	×20.75=	+ 2.93 Cal.
Water	−3.6163 kg.	×1.0	×20.75=	−75.04 Cal.
Irregular excretion of dry matter	0.2334	×0.4	×20.75=	+ 1.93 Cal.
Correction for gain by body				−69.53 Cal.

It will be noted that the average body temperature of the animal has been used, ignoring the small daily fluctuations. For the specific heats of protein and fat, Rosenthal's values (*9*) have been applied. In the experiments with steers variations in the dry matter excreted per day were relatively small, and this item was neglected in computing the thermal effect correction. However, in these experiments the variations were so great as to require correction as shown above.

Since the nitrogen contained in the urine represents incompletely oxidized protein it is necessary to bring the energy balance to a computed state of nitrogen equilibrium in order to arrive at a true value for the metabolizable energy. This has been done in Table XV. The method of computation followed is the same as in previous publications from this institute.

TABLE XV.—*Energy of urine, and of protein gained or lost, corrected to nitrogen equilibrium*

Experiment, animal, and period Nos.	Energy of urine uncorrected for gain or loss of nitrogen *	Gain of N by body	Correction N×7.45	Corrected energy	Energy of protein uncorrected	Correction, protein ×5.7	Corrected energy of protein
	Cals.	*Grams*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221D–885–I	6,595.0	−7.5	−55.9	6,539.1	256.5	−55.9	200.6
II	9,660.1	+4.7	+35.0	9,695.1	160.7	−35.0	125.7
III	6,508.7	+2.7	+20.1	6,528.8	92.3	−20.1	72.2
221D–886–I	6,681.8	+6.0	+44.7	6,696.5	205.2	−44.7	160.5
II	10,569.7	+4.2	+31.3	10,601.0	143.6	−31.3	112.3
III	6,517.1	+5.6	+41.7	6,558.8	191.5	−41.7	149.8
221E–885–I	8,045.0	+17.3	+128.9	8,173.9	591.7	−128.9	462.8
II	5,991.8	+3.3	+24.6	6,016.4	112.9	−24.6	88.3
221F–874–I	10,636.7	+2.8	+20.9	10,657.6	95.8	−20.9	74.9
II	7,265.3	+1.0	+7.5	7,272.8	34.2	−7.5	26.7
221F–887–I	9,180.7	+4.3	+32.0	9,212.7	147.1	−32.0	115.1
II	6,236.7	−4.9	−36.5	6,200.2	167.6	−36.5	131.1

* Corrected for loss on drying.

If there is a gain of protein by the body, then nitrogen is stored which would have appeared in the urine had actual nitrogen equilibrium been attained. In this case the correction for nitrogen is added to the energy of the urine. In case there is a loss of body protein there appears in the urine nitrogen derived from the body tissue and not from the feed. Therefore the correction is subtracted.

Having thus brought the body to nitrogen equilibrium by correcting the energy of the urine, and therefore the metabolizable energy, it becomes necessary to apply a corresponding correction to the computed energy equivalent of the protein stored or lost. This has been done in the last three columns of Table XV. It will be noted that the energy correction for the protein is always subtracted, because in computing the energy equivalent of the protein the full heat value (heat of combustion) of the protein is used, while in reality a portion of the protein energy is not utilizable, but appears in the urine in the form of incompletely oxidized nitrogenous compounds. In the case of a loss of protein, the energy equivalent is abstracted from the body and does not enter into consideration of the feed.

THE BALANCE OF ENERGY

From the data recorded on the preceding pages the balance of energy has been computed and the results set forth in Table XVI.

TABLE XVI.—*The balance of energy per head and day*

	221D			221D		
	885–I	885–II	885–III	886–I	886–II	886–III
	Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Income	16,843.9	25,057.3	17,808.9	17,503.5	27,867.4	17,492.7
Outgo	7,855.9	11,583.2	8,172.6	8,242.5	12,735.0	8,127.9
Metabolizable	8,988.0	13,474.1	9,636.3	9,261.0	15,132.4	9,364.8
Heat	8,380.3	9,607.4	8,816.7	8,058.6	10,474.5	7,914.1
Gain by body:						
Protein*	−200.6	+125.7	+72.2	+160.5	+112.3	+149.8
Fat	+987.1	+4,249.4	+274.6	+692.6	+4,245.6	+704.0
Error	−178.8	−508.4	+472.8	+349.3	+300.0	+596.9

	221E		221F		221F	
	885–I	885–II	874–I	874–II	887–I	887–II
	Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Income	21,514.9	15,727.3	27,577.9	18,460.5	24,953.3	16,705.5
Outgo	9,860.7	7,462.2	12,819.0	8,863.2	11,317.0	7,695.5
Metabolizable	11,654.2	8,265.1	14,758.9	9,597.3	13,636.3	9,010.0
Heat	9,721.7	8,735.5	10,964.2	9,076.8	10,327.2	8,395.0
Gain by body:						
Protein*	+462.8	+88.3	+74.9	+26.7	+115.1	−131.1
Fat	+2,034.0	−267.9	+3,404.8	+125.4	+2,871.9	+407.6
Error	−564.3	−290.8	+315.0	+368.4	+322.1	+338.5

* Corrected for nitrogen gained or lost by the body.

Considering now cow 885, of whose erratic behavior more will be said in subsequent pages, there was a net gain of energy by the body in each of the periods of experiment 221D. Since Periods I and II were designed to approximate maintenance the gain was slight, and

in Period I a loss of protein occurred. The ration fed in Period III was a duplication of that fed in Period I. In each there was a small gain of energy, differently distributed, however, between protein and fat. There would seem to be two plausible explanations for this difference—the influence of previous feeding (a supermaintenance ration) on Period III, or the refusal of considerable quantities of feed in Period I. The writers are inclined to give the greater weight to the latter possibility.

In the lower half of this table appears an item "error." In effect, this represents the difference between the computed and the observed heat measurements. If this error is assigned to the metabolizable energy it represents but a small percentage of the total. If it were included in the heat production its effect would be slightly increased but would still be relatively small. On the other hand, if it were considered as a part of the energy equivalent of the body gain it becomes a major factor materially affecting subsequent computations.

It is probable, however, that this item of error is a composite of a number of components of various origins. However, taking into account the painstaking attention to detail and the minute checking of the individual measurements in connection with the operation of the respiration calorimeter, and, on the other hand, the assumption that the dry matter gained or lost by the body consists of protein and fat alone, and the influence of the loss on air drying the feces on the value determined for the metabolizable energy, it would seem that the observed heat emission must be the most accurate. Therefore it is considered as the reference base in the subsequent treatment of the experimental data.

In the case of cow 886 there were also two periods (I and II) on a maintenance ration, and one period (III) on supermaintenance. In general, the results are similar to those obtained with cow 885. There is a better agreement between Periods I and III, but the "error" is considerably greater in the latter period. Judging from the appearance of the two animals, and comparing their individual reaction to the imposed experimental conditions, we should expect cow 886 to make the more efficient use of her feed. That this was actually the case is shown by a comparison of the experimental data for the two animals.

In 1921, cow 885, which had been bred immediately following her last experimental period in 1920, apparently was with calf and was so reported by the barn attendants. However, prior to the beginning of the next experimental period, she was found to be farrow. While her weight had increased since the preceding year, Table II shows the gain to have been small in relation to the feed received. Yet she appeared to be fat. She was extremely restless. Her neck became noticeably thick, as sometimes occurs in cows which have become barren. Examination by a veterinarian failed to reveal definite functional disorder. It was therefore decided to carry her along in experiment 221E. The results obtained with this cow in the maintenance period (II) are markedly unlike those obtained in the corresponding period of experiment 221D. No definite reason is apparent other than error due to the refusal of feed during all experimental periods, which, of course, renders doubtful the significance of the data obtained with the respiration calorimeter. This

cow was slaughtered after the close of experiment 221E, and, although the quantity of fat on the internal organs was unusually great, she appeared to be sound. The data obtained with this cow have been carried throughout the computations, but on account of the question as to their significance have not been included in averages.

The data presented for the two cows in experiment 221F require no special discussion. They are apparently normal in every respect.

It will be noted that the gains of energy by the body in all the maintenance periods are small, and it is assumed that they will not adversely affect the computations of net-energy values for maintenance.

In two maintenance periods, 221D–885–I and 221F–887–II, there was a slight loss of body protein. In the case of cow 885 there was a slight gain of protein in Period III, which was designed to duplicate Period I.

THE OBSERVED AND COMPUTED HEAT PRODUCTION

In Table XVII data are collected for comparison of the observed and computed heat production as obtained in these experiments. Excluding the data for cow 885, which are questionable, the agreement is reasonably good. The effect of the refused feed in the case of cow 885 is clearly apparent in the data presented in this table, and, as will be shown in the computation of the net-energy values, the heat increment per kilogram of the feed is much too low. As is shown in an analysis of the subject by Max Kriss (*6*) the possible sources of error in the observed as compared with the computed heat production are as indicated in the discussion following Table XVII.

TABLE XVII.—*Observed and computed daily heat production*

Experiment, animal, and period Nos.	Observed	Computed	Error	$\frac{\text{Computed}}{\text{Observed}} \times 100$
	Cals.	*Cals.*	*Cals*	*Per cent*
221D–885–I	8,380.3	8,201.5	−178.8	97.87
II	9,607.4	9,099.0	−508.4	94.71
III	8,816.7	9,289.5	+472.8	105.36
221D–886–I	8,058.6	8,407.9	+349.3	104.33
II	10,474.5	10,774.5	+300.0	102.86
III	7,914.1	8,511.0	+596.9	107.54
221E–885–I	9,721.7	9,157.4	−564.3	94.20
II	8,735.5	8,444.7	−290.8	96.67
221F–874–I	10,964.2	11,279.2	+315.0	102.87
II	9,076.8	9,445.2	+368.4	104.06
221F–887–I	10,327.2	10,649.3	+322.1	103.12
II	8,395.0	8,733.5	+338.5	104.03

The factors entering into the observed heat production, and therefore effecting the net-energy values, are (1) the heat emission by radiation and conduction; (2) the heat emission as latent heat of water vapor; (3) the relative time spent in the standing position as compared with that spent lying; (4) the refusal of feed; (5) the gain or loss of matter by the body; and (6) the thermal environment.

In connection with the computed heat production there enter into the writers' computations the heat of combustion of the feed, of the excreta, of the methane, and of the shed hair and scurf, together

with the necessary allowances of thermal energy for fat and protein gained or lost by the body.

Considering these factors seriatim, the heat emission by radiation and conduction, constituting about 75 per cent of the total heat emission, is accurately determined; the estimation of the heat emission as latent heat of water vapor somewhat less so. Check tests by Armsby and Fries (*1, p. 217–222*) place the possible error of this latter determination at 6 per cent. This may be taken as the extreme, since the quantity of water vapor liberated in the check tests with alcohol is so much smaller than that measured in experiments with cows. The influence on the observed heat production of standing as compared with lying is an important factor. Animals vary greatly in the relative time spent standing and lying, and, on account of the greater energy cost of maintaining the animal in the standing position, it is necessary, in determining net-energy values of feeds or net-energy requirements of animals, to eliminate the influence of this varible. This point is treated in the discussion following Table XIX.

The influence of refusal of feed on the observed heat production is shown by the data for cow 885. The refusal of feed was about at a maximum just prior to the measurement of the heat emission in the respiration calorimeter. Hence the effect is much magnified.

The gain or loss of matter by the body influences the heat emission, in that heat is required to raise any gain from the temperature of the chamber to the temperature of the body; and, conversely, in the case of loss of body substance, heat is liberated through the cooling of the lost materials from the temperature of the body to that of the chamber.

The thermal environment would of course have a very definite influence upon the heat emission, but so long as it is maintained above the critical temperature for the subject of the investigation no error can be introduced from this source. In practical husbandry, the keeping of animals below the critical temperature constitutes a special case to which the net-energy values as determined here do not apply. It must be kept in mind, however, that the point at which physical regulation of body tempetature gives way to chemical regulation is not fixed and unvarying, but is affected by the amount of the feed eaten. This becomes very important in connection with measurements of heat production on submaintenance rations and in experiments involving actual fasting.

In the case of the computed heat production some of the factors enumerated as affecting the observed heat production, notably the relative time spent standing as compared with lying, the refusal of feed, and the thermal environment, also apply. There are, however, other factors which affect only the computed heat production. These relate principally to the accuracy with which the heat of combustion of the feed and of the excreta is determined. The methane produced also becomes a material factor, as do the protein and fat gained or lost by the body. These latter considerations depend of course on the accurate determination of carbon and nitrogen; and the writers in their efforts to account for the difference between the observed and computed heat production found that very small variations in the carbon estimations are so multiplied

in the course of the computations as to materially affect the energy equivalent to the fat gained, and through this the net-energy values determined by the indirect method.

Careful consideration of the items enumerated and comparison of the available data leads to the conclusion that the observed heat production as determined here is the more accurate, but that the computed heat production may be used in the determination of net-energy values without significant compromise.

In Table XVIII the data relative to the metabolizable energy have been collected for ready reference in connection with the subsequent computation of the net energy value of the ration and the maintenance requirements of the cows.

TABLE XVIII.—*Metabolizable energy per kilogram of dry matter of rations*

Experiment, animal, and period Nos.	Dry matter eaten	Total metabolizable energy	Metabolizable energy per kilogram dry matter
	Kg.	*Cals.*	*Cals.*
221D–885–I	3.6674	8,988.0	2,450.8
II	5.4676	13,474.1	2,464.4
III	3.8505	9,636.3	2,502.6
Total and average	12.9855	32,098.4	2,471.9
221D–886–I	3.8131	9,261.0	2,428.7
II	6.0809	15,132.5	2,488.5
III	3.7821	9,364.8	2,476.1
Total and average	13.6761	33,758.2	2,468.4
221E–885–I	4.7012	11,654.2	2,479.0
II	3.4475	8,265.1	2,397.4
Total and average	8.1487	19,919.3	2,444.5
221F–874–I	5.9803	14,758.9	2,467.9
II	4.0032	9,597.3	2,397.4
Total and average	9.9835	24,356.2	2,439.6
221F–887–I	5.4183	13,636.3	2,516.7
II	3.6274	9,010.0	2,483.9
Total and average	9.0457	22,646.3	2,503.5

THE NET-ENERGY VALUE OF THE RATION

Only a part of the metabolizable energy of a ration is utilizable by the body. That part which is utilized can be determined, however, and it is a true measure of the actual value of the feed to the animal, from the energy standpoint. To this available portion of the metabolizable energy the term net energy is applied.

In the following computations, which are based upon the heat production of the subject of the experiment, the heat production as measured has been computed to the standard day of 12 hours standing and 12 hours lying, using the method outlined by Fries and Kriss (*5*) in their recent discussion of the influence of standing and lying on the metabolism of cattle. The results of this computation are presented in Table XIX.

TABLE XIX.—*The observed heat production corrected to a standard day of 12 hours standing and 12 hours lying*

Experiment, animal, and period Nos.	Total heat production	Time standing		Live weight	Correction of heat production for standing		Heat production corrected to 12 hours standing
		Total per day	Difference from 12 hours		Per hour	Total	
	Cals.	*Hours*	*Hours*	*Kg.*	*Cals.*	*Cals.*	*Cals.*
221D-885-I	8,380.3	17.7	−5.7	415.2	27.4	−156.2	8,224.1
II	9,607.4	16.1	−4.1	429.7	27.4	−112.3	9,495.1
III	8,816.7	17.8	−5.8	426.4	27.4	−158.9	8,657.8
221D-886-I	8,058.6	11.0	+1.0	399.8	26.3	+26.3	8,084.9
II	10,474.5	9.2	+2.8	424.4	27.4	+76.7	10,551.2
III	7,914.1	7.9	+4.1	420.2	27.4	+112.3	8,026.4
221E-885-I	9,721.7	19.4	−7.4	443.1	28.5	−210.9	9,510.8
II	8,735.5	18.3	−6.3	434.4	27.4	−172.6	8,562.9
221F-874-I	10,964.2	11.9	+0.1	428.8	27.4	+2.7	10,966.9
II	9,076.8	11.4	+0.6	415.6	27.4	+16.4	9,093.2
221F-887-I	10,327.2	17.3	−5.3	335.2	22.9	−121.4	10,205.8
II	8,395.0	17.3	−5.3	320.5	22.9	−121.4	8,273.6

THE INCREMENT OF HEAT PRODUCTION DUE TO FEED CONSUMPTION

In the method evolved by Armsby the determination of the net energy of the feed calls for the measurement of the increase in heat production resulting from the addition of a known quantity of feed to a basal ration. To this increase in heat production the term "heat increment" has been applied. The data expressing the heat increments in this study are recorded in Table XX.

TABLE XX.—*The increment of heat production due to feed consumption, computed to the standard day*

Experiment, animal, and period Nos.	Dry matter eaten	Heat production, observed
	Kg.	*Cals.*
221D-885-II	5.4676	9,495.1
I	3.6674	8,224.1
Difference	1.8002	1,271.0
Difference per kg. dry matter		706.0
221D-885-II	5.4676	9,495.1
III	3.8505	8,657.8
Difference	1.6171	837.3
Difference per kg. dry matter		517.8
Average for cow 885		611.9
221D-886-II	6.0809	10,551.2
I	3.8131	8,084.9
Difference	2.2678	2,466.3
Difference per kg. dry matter		1,087.5
221D-886-II	6.0809	10,551.2
III	3.7821	8,026.4
Difference	2.2988	2,524.8
Difference per kg. dry matter		1,098.3
Average for cow 886		1,092.9
221E-885-I	4.7012	9,510.8
II	3.4475	8,562.9
Difference	1,2537	947.9
Difference per kg. dry matter		756.1
221F-874-I	5.9803	10,966.9
II	4,0032	9,093.2
Difference	1.9771	1,873.7
Difference per kg. dry matter		947.7
221F-887-I	5.4183	10,205.8
II	3.6274	8,273.6
Difference	1.7909	1,932.2
Difference per kg. dry matter		1,078.9

The data obtained in the experimental work with cow 885 have been included in Table XX, notwithstanding their obvious unreliability. It is plainly evident that the heat production of this cow is not truly related to the feed of the period, and has been rendered valueless for present purposes by the refusal of feed while on experiment. As specific evidence on this point, attention is directed to experiment 221D–885–II–III. The computed heat production in the maintenance period (III), Table XVII, is greater than in the supermaintenance period (II), an absurd finding under the conditions of feed intake and environmental temperature of the experiment.

Referring to Table XX, omitting the data obtained with cow 885, the average heat increment per kilogram of dry matter of the ration is 1,053.1 Calories.

THE COMPUTED FASTING KATABOLISM AS A MEASURE OF THE MAINTENANCE REQUIREMENT

It must be kept clearly in mind that the term fasting katabolism is not synonymous with basal katabolism, which latter involves the element of complete muscular repose. The term "fasting katabolism," as used in this paper, represents the minimum energy requirement incident to the normal daily activity of the animal, and might more accurately be termed the "24-hour normal fasting katabolism." It will also be noted that in computing the maintenance requirement to a 12-hour-standing basis, this view has been modified to some extent, for it is impossible to compare the maintenance requirements of two animals one of which, for instance, normally stands three-fourths of the time and the other one-half of the time without neutralizing the effect of this variant.

The importance of the 24-hour normal fasting katabolism in relation to feeding practice is obvious. While it is not as nearly constant as the theoretical true basal katabolism, its use is rendered imperative in experiments involving animals the muscular movements of which can not be perfectly controlled.

The foregoing data provide a basis for computing the maintenance requirement of the individual animal. The relatively constant total katabolism of a fasting animal (above the point of critical thermal environment) is a measure of the energy required for vital activities, and is consequently the minimum amount required for maintenance. Since it has been considered impracticable heretofore to measure the normal fasting katabolism of cattle directly by complete fasting experiments, the result sought has been attained indirectly from experiments in which the subjects received certain amounts of feed, as expressed in Table XXI.

TABLE XXI.—*The net energy required for maintenance per head, computed to the standard day*

Experiment, animal, and period Nos.	Dry matter eaten	Based on observed heat production *			
		Heat increment per kilogram dry matter	Total heat increment	Heat production	Net energy for maintenance
	Kg.	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221D-885-I	3.6674	611.9	2,244.1	8,224.1	5,980.0
II	5.4676		3,345.6	9,495.1	6,149.5
III	3.8505		2,356.1	8,657.8	6,301.7
Average					6,143.7
221D-886-I	3.8131	1,092.9	4,167.3	8,084.9	3,917.6
II	6.0809		6,645.8	10,551.2	3,905.4
III	3.7821		4,133.5	8,026.4	3,892.9
Average					3,905.3
221E-885-	4.7012	756.1	3,554.6	9,510.8	
II	3.4475		2,606.7	8,562.9	
Average					5,956.2
221F-874-I	5.9803	947.7	5,667.5	10,966.9	
II	4.0032		3,793.8	9,093.2	
Average					5,299.4
221F-887-I	5.4183	1,078.9	5,845.8	10,205.8	
II	3.6274		3,913.6	8,273.6	
Average					4,360.0

* Heat production corrected to standard day of 12 hours standing and 12 hours lying.

It will be noted that the writers' method of computation of the fasting katabolism is as given by Armsby (*3, p. 282, sect. 374, par. 2*). This method should find general use, because it eliminates from consideration the gain or loss of energy by the body and does not make use of the metabolizable energy directly. It further serves to make clear the relationship existing between the net-energy value of the feed, the fasting katabolism, and the maintenance requirement.

The fasting katabolism has been determined for these three animals through a comparison of the effect produced by two different quantities of the same feed. The essential data involved are included in Table XXI.

The values given in Table XXI, represent the maintenance requirements of these cows for a standard day of 12 hours standing and 12 hours lying. The data are not strictly comparable, however, because the differences in live weight have not been taken into account. For comparative purposes, therefore, the values given in Table XXI for the maintenance requirement of the experimental subjects have been computed to uniform live weights of 500 kg. and 1,000 pounds, these data comprising Table XXII. The data for cow 885 have not been so computed because of their doubtful accuracy.

TABLE XXII.—*The net energy required for maintenance computed to the standard day and to uniform live weight*

Experiment, animal, and period Nos.	Live weight	Net energy for maintenance		
		As determined, observed	Per 500 kilograms live weight [a] observed	Per 1,000 pounds live weight [a] observed
	Kg.	*Cals.*	*Cals.*	*Cals.*
221D–886–I	399.8	3,917.6	4,547.8	4,267.0
II	424.4	3,905.4	4,357.0	4,088.0
III	420.2	3,892.9	4,370.9	4,101.1
Average	414.8	3,905.3	4,425.2	4,152.0
221F–874–I	422.2	5,299.4	5,932.1	5,565.9
II				
221F–887–I	327.9	4,360.0	5,776.7	5,420.0
II				

[a] Computed in proportion to the two-thirds power of the live weight.

That there should be material variation in the maintenance requirements of these three animals is to be expected from their behavior under the experimental conditions. Cow 886 was extremely quiet, was not restless when the attendants were working around her, and spent more than half of the experimental time in the lying position. Cow 874 also spent about half her time lying quietly, but when standing was much more restless. Cow 887 stood the greater part of the time, and while in the respiration calorimeter was addicted to rubbing vigorously against the side of the stall, and while lying would shift suddenly from one side to the other without arising.

In interpreting the figures given as representing the maintenance requirements of the subjects of this investigation, it must be kept in mind that direct comparison of the individuals, one with the other, is possible only after the data have been reduced to a common basis as regards standing and lying, and to a uniform live weight. This having been done, then the variations observed are due to individual differences in the animals studied.

THE NET ENERGY REQUIRED FOR MAINTENANCE COMPUTED TO THE STANDARD DAY AND TO UNIFORM LIVE WEIGHT

The average net energy required for maintenance of each of the three cows, as directly observed, and expressed in therms per 500 kg. live weight (Table XXII) is: Cow 886, 4.424 therms; cow 874, 5.932 therms; cow 887, 5.777 therms. The values as determined are not complicated by the requirements of fetal growth, since cow 886 was but two months pregnant at the beginning of the last experimental period of which she was the subject, and cows 874 and 887 had not been bred.

In experiment 221F there were two periods only with each cow. On this account the values given for the maintenance requirement of cows 874 and 887 must be taken as reresentative of the average of the live weights of the two periods. The difference in live weight is small in each case and has no material influence on the determined values.

THE NET-ENERGY VALUE OF THE RATION

In computing the net-energy value of the ration the writers have followed the improved procedure of Kriss (7). Table XXIII contains the data necessary to arrive at the total net energy of the ration. The items for energy gained are obtained by subtracting the heat production (computed to the standard day) from the metabolizable energy of the feed.

TABLE XXIII.—*The gains of energy by the animals and the total energy of the ration*

Experiment, animal, and period Nos.	Metabolizable energy	Based on observed heat production *			
		Observed heat production	Energy gained	Net energy for maintenance	Total net energy of ration
	Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*
221D–886–I	9, 261. 0	8, 084. 9	+1, 176. 1	3, 905. 3	5, 081. 4
II	15, 132. 4	10, 551. 2	+4, 581. 2	3, 905. 3	8, 486. 5
III	9, 364. 8	8, 026. 4	+1, 338. 4	3, 905. 3	5, 243. 7
221F–874–I	14, 758. 9	10, 966. 9	+3, 792. 0	5, 299. 4	9, 091. 4
II	9, 597. 3	9, 093. 2	+504. 1	5, 299. 4	5, 803. 5
221F–887–I	13, 636. 3	10, 205. 8	+3, 430. 5	4, 360. 0	7, 790. 5
II	9, 010. 0	8, 273. 6	+736. 4	4, 360. 0	5, 096. 4

* Heat production computed to standard day of 12 hours standing and 12 hours lying.

Tables XXIV and XXV present the net-energy value of the ration, as determined with three dry cows, expressed in terms of percentage utilization of the metabolizable energy, and as per kilogram dry matter of the ration. Cow 885 has been excluded from consideration on account of the persistent refusal of feed, which has been shown to vitiate the values found.

TABLE XXIV.—*The percentage utilization of the metabolizable energy of the ration*

Experiment, animal, and period Nos.	Metabolizable energy	Based on the observed heat production *	
		Total net energy of ration	Percentage utilization of the metabolizable energy
	Cals.	*Cals.*	*Per cent*
221D–886–I	9, 261. 0	5, 081. 4	54. 9
II	15, 132. 4	8, 486. 5	56. 1
III	9, 364. 8	5, 243. 7	56. 0
221F–874–I	14, 758. 9	9, 091. 4	61. 6
II	9, 597. 3	5, 803. 5	60. 5
221F–887–I	13, 636. 3	7, 790. 5	57. 0
II	9, 010. 0	5, 096. 4	56. 6

* Heat production computed to standard day of 12 hours standing and 12 hours lying.

TABLE XXV.—*The net energy per kilogram of dry matter of the ration*

Experiment, animal, and period Nos.	Dry matter eaten	Based on the observed heat production [a]	
		Total net energy of ration	Net energy per kilogram dry matter
	Kg.	*Cals.*	*Cals.*
221D-886-I	3.8131	5,081.4	1,332.6
II	6.0809	8,486.5	1,395.6
III	3.7821	5,243.7	1,386.5
221F-874-I	5.9803	9,091.4	1,520.2
II	4.0032	5,803.5	1,449.7
221F-887-I	5.4183	7,790.5	1,437.8
II	3.6274	5,096.4	1,405.0

[a] Heat production computed to standard day of 12 hours standing and 12 hours lying.

The percentages of utilization of metabolizable energy given in Table XXIV show good agreement. The maximum is 61.6 per cent, the minimum 54.9 per cent, and the average for the series 57.7 per cent.

It would appear from these data that individual differences exist in the expense of utilization of the metabolizable energy. These differences represent unlike energy expenditure, on various accounts, in the utilization of the feed, and also any such error as there may be in the determination of the maintenance requirement, the metabolizable energy, and the heat increment.

The experimental animals were apparently normal, average individuals, and the data for percentage utilization seem to satisfactorily represent the net energy of the ration as a proportion of that which is metabolizable.

The data presented in Table XXV represent the net-energy value of the ration expressed as Calories per kilogram of dry matter of the feed. The average value found is 1,418.2 Calories.

In determining the rations to be fed during this series of experiments, the net-energy value of the ration was calculated from the average values for the hay and grain components as given by Armsby. (*2*) These data follow:

	Therms per kg. dry matter.
Oats	1.637
Corn meal	2.113
Wheat bran	1.297
Linseed meal	2.152
Mixed grain	1.730
Alfalfa hay	0.824
In ration used	1.364

The value obtained in experiment 221F-874-I (Table XXV) seems to be high, but no valid reason can be found for excluding it. Comparing the average value obtained in this series of experiments with that computed from Armsby's average values, the former is 4 per cent higher.

SUMMARY

The net energy required for maintenance by three dry cows was determined in a series of respiration calorimeter experiments to be 4.150, 5.420, and 5.566 therms, respectively, per 1,000 pounds of live weight. The lowest value (4.150 therms) is ascribed to the individuality of the subject, which was a cow of unusually quiet disposition. The other two cows appeared to be in no way unusual, and their maintenance requirements are not abnormally low, though appreciably less than Armsby's published average of 6 therms. It would seem, therefore, that this standard figure is sufficiently high.

Since two of the three determinations of maintenance requirement fall within the range of variation of the previous figures for steers published from the institute, there is in these results no definite warrant for anticipating the establishment of a maintenance requirement for cows differing from that of steers.

The applicability of these results to feeding under conditions of practice is indicated by the fact that the average maintenance requirement of 6 therms of energy for steers, as determined at the institute, has been found to be a satisfactory measure of the maintenance requirement, under conditions of practice, not only of steers but also of cows.

In these experiments there were gains of energy by all three subjects on rations computed to supply the maintenance requirements in accord with the 6-therm average.

The net-energy value of a ration composed of 40 per cent alfalfa hay and 60 per cent grain mixture, the latter consisting of 30 parts wheat bran, 30 parts ground oats, 30 parts corn meal, and 10 parts old process linseed meal, was found to be 1.418 therms per kilogram of dry matter of the ration, as determined by direct measurement of the heat production of the animals.

A method of approximating the apparent digestibility of a ration where it is impracticable to collect the dung and urine separately is reported.

LITERATURE CITED

(1) ARMSBY, H. P., and FRIES, J. A.
1911. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 128, 245 p., illus.

(2) ———
1912. THE MAINTENANCE RATIONS OF FARM ANIMALS. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 143, 110 p., illus.

(3) ———
1917. THE NUTRITION OF FARM ANIMALS. 743 p., illus. New York.

(4) FRIES, J. A., BRAMAN, W. W., and COCHRANE, D. C.
1924. RELATIVE UTILIZATION OF ENERGY IN MILK PRODUCTION AND BODY INCREASE OF DAIRY COWS. U. S. Dept. Agr. Bul. 1281, 36 p., illus.

(5) ——— and KRISS, M.
1924. METABOLISM OF CATTLE DURING STANDING AND LYING. Amer. Jour. Physiol. 71: 60–83.

(6) KRISS, M.
1925. A COMPARISON OF DIRECT AND INDIRECT CALORIMETRY IN INVESTIGATIONS WITH CATTLE. Jour. Agr. Research 30: 393–406.

(7) ———
1925. AN IMPROVED METHOD OF COMPUTATION OF NET-ENERGY VALUES OF FEEDING STUFFS. Jour. Agr. Research 31: 469–484.

(8) RICHARDS, E. H.
1917. THE FIXATION OF NITROGEN IN FAECES. Jour. Agr. Sci. 8: 299–311, illus.
(9) ROSENTHAL, I.
1878. UEBER DIE SPECIFISCHE WÄRME THIERISCHER GEWEBE. Arch. Anat. u. Physiol., Physiol. Abt. 1878: 215–217.
(10) TROWBRIDGE, P. F., MOULTON, C. R., and HAIGH, L. D.
1915. THE MAINTENANCE REQUIREMENT OF CATTLE. Mo. Agr. Exp. Sta. Research Bul. 18, 62 p., illus.

REVISED NET-ENERGY VALUES OF FEEDING STUFFS FOR CATTLE[1]

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INTRODUCTION

One of the chief purposes of the work with the respiration calorimeter at the Pennsylvania Institute of Animal Nutrition has been to determine the total expenditure of energy by animals in the utilization of feed for maintenance and production, the difference between the gross energy and the energy losses and expenditures of utilization constituting the net-energy value.

The methods employed and the results obtained with steers, during the years 1902 to 1915, have been reported in articles by Armsby and Fries (*1*, *2*, *3*, *4*, *6*, *7*, *8*, *9*).[2] The derivation of these values involves comparisons of heat production in periods of unlike feed consumption, these comparisons being dependent upon uniform conditions as to expenditure of energy for maintenance, especially as determined by the time spent by the animal in the standing and the lying positions.

In recent studies at this institute (*10*, *11*), the evidence obtained has disclosed imperfections in the treatment of the experimental data and in the computations which have led to the published net-energy values. These studies have led (1) to a new method of correcting the heat production of the experimental animal to a standard day, as to standing and lying, and (2) to an important change in the method of computation of the net-energy values. These improved procedures, which have been described in detail elsewhere (*10*, *11*), are of such importance as to necessitate the recalculation of all of the net-energy determinations of feeds for steers which have been published from this institute, and the corrected results are set forth in this paper.

EXPERIMENTS AND ANIMALS

The experiments involved in this recomputation include 12 series, comprising 71 experimental periods, with 9 different steers, varying in age from 20 months to approximately 60 months at the beginning of the several experiments.

This covers all of the net-energy values of feeds for steers which have been determined at this institute (*1*, *2*, *3*, *4*, *6*, *7*, *8*, *9*), except the results of two series of experiments (Nos. 190 and 208) with young, growing steers, which have been omitted from this paper for reasons which will be explained.

Data relating to the steers which served as experimental subjects in these investigations comprise Table I.

[1] Received for publication Feb. 18, 1925; issued January, 1926.

[2] Reference is made by number (italic) to "Literature cited" p. 1098.

TABLE I.—*Descriptive data relating to the experimental subjects*

Animal	Breed	Number of experiments in which used	Age at beginning of experiment	Average live weight
			Months	*Kg.*
I	Grade Shorthorn	174	36	408
		179	48	528
		186	60	572
A	Aberdeen Angus	200	23	408
		207	35	510
B	Scrub	200	25	303
		207	37	380
D	Grade Hereford	210	21	331
		211	33	449
F	do	209	21	300
G	Hereford	211	28	379
H	Shorthorn	212	20	345
J	do	216	22	383
		217	33	512
		217	37	649
K	do	220	24	501

NEW METHOD OF CORRECTING THE HEAT PRODUCTION TO A STANDARD DAY OF 12 HOURS STANDING AND 12 HOURS LYING

The question of the influence of position on the metabolism of cattle has been studied and discussed in many publications by Armsby and Fries (*3*, *4*, *5*, *6*), who devised and used a method of correcting the heat production to a standard day, as to standing and lying, based on the rate of heat elimination as directly measured in these different positions. A recent critical study of this problem by Fries and Kriss (*10*) has revealed this method to be seriously in error, first, because of the inaccurate division of the heat between the intervals of standing and lying, due to several factors, but principally to the large capacity of the apparatus to store heat while the animal is lying and to radiate this heat during the subsequent period of standing, and, second, because this method, in effect, involves the assumption that the energy required for maintenance and for feed utilization contribute either at uniform or at characteristic rates to the total heat production of standing and of lying, and are equally affected by lapse of time spent in these positions.

This study has led not only to the discovery and estimation of the instrumental errors, but also to a change of conception of the causes for increased heat production while standing as compared with lying, and to the formulation of a new and simple method of correcting the daily heat production, as measured, to a standard day as to standing and lying.

That an animal should produce more heat while standing than while lying, other conditions remaining equal, is readily understandable on the basis of greater muscular activity in the standing position. This excess of heat, which represents a part of the maintenance requirement of net energy, was found with a fasting dry cow weighing 400 kg. to be 26.3 Cals. per hour.

With this figure as a basis we correct the heat production, as measured, to represent a standard day of 12 hours standing and 12 hours lying, thus establishing a basis for determining the difference

in heat production between periods compared, in relation to the differences in feed. This factor (26.3 Cals. per hour), representing an animal of 400 kg. live weight, is modified for animals of other weights in accord with the two-thirds power of the live weight, as follows:

Live weight	Factor	Live weight	Factor	Live weight	Factor	Live weight	Factor
Kg.	*Cals.*	*Kg.*	*Cals.*	*Kg.*	*Cals.*	*Kg.*	*Cals.*
300	21.7	375	25.2	450	28.5	525	31.5
325	22.9	400	26.3	475	29.5	550	32.5
350	24.1	425	27.4	500	30.5	575	33.5

The correction to the standard day of 12 hours standing and 12 hours lying is computed by determining the difference in hours between 12 (the standard) and the time actually spent in the standing position, and multiplying this difference by the factor corresponding to the live weight of the animal. This correction is subtracted from the heat production as measured, or is added thereto, according to whether the animal stood more or less than 12 hours during the calorimeter day. For example, the daily heat production of a steer weighing 501 kg. was 10,754 Cals. The steer spent 9.25 hours per day in the standing position. The correction for standing is $(12-9.25)\times 30.5=+84$ Cals., and the heat production corrected to the standard day of 12 hours standing and 12 hours lying is $10{,}754+84=10{,}838$ Cals. If this animal had stood 14.75 hours per day the correction would be $(12-14.75)\times 30.5=-84$ Cals., and to correct the daily heat production to the standard day of 12 hours standing and 12 hours lying the 84 Cals. would be subtracted. It should be noted that for the determination of the heat increment caused by the feed, and, consequently, for the determination of the net-energy value of the feed, the length of the standard day is immaterial. For a detailed discussion of this method and the derivation of the factors, the reader is referred to the original publication (*10*).

DATA FOR THE DETERMINATION OF NET-ENERGY VALUES

In order to compute the net-energy value of a feeding stuff, it is necessary to have from each of two or more periods of differing intake of the feed of interest, (1) the daily dry matter of the feed eaten, (2) the metabolizable energy of the ration (energy of the feed minus the energy of the excreta), and (3) the daily heat production by the animal, corrected to the standard day of 12 hours standing and 12 hours lying.

In recomputing the net-energy values of the feeds for which such values have been published from this institute, the data for dry matter of feed, for metabolizable energy, and for the daily heat production, as measured, remain as previously reported, except where arithmetical or other errors of work have been discovered;[3] but inasmuch as the new method of correcting the heat production to a

[3] Attention is called to an error in the value for the average metabolizable energy per kilogram of dry matter of timothy hay in experiment 174 as reported in the following: H. P. Armsby and J. A. Fries, Net energy values of feeding stuffs for cattle, Jour. Agr. Research 3: 441, 444, 1915. The value reported there is 1,674 Cals. The correct value is 1,953 Cals. For the linseed meal in this experiment the writers used the values for metabolizable energy, heat increment, and net energy given in the following: H. P. Armsby, The nutrition of farm animals, 743 p., illus., New York, 1917.

standard day differs from that previously used, the data for the "corrected" heat production differ, of course, from those which have been published. In order to make it possible to follow the recomputation of the net-energy values the complete data are presented in Table II. The gains of energy given in the last column of the table are computed by subtracting the corrected heat production from the metabolizable energy.

TABLE II.—*Data for computation of net-energy values*

Experiment No. and feeding stuffs	Animal	Period	Time spent standing per day	Dry matter eaten		Metabolizable energy of ration	Heat production corrected to 12 hours standing	Gain of energy by animal
				Roughage	Concentrates			
			Hours	*Kg.*	*Kg.*	*Cals.*	*Cals.*	*Cals.*
174. Timothy hay and linseed meal	I	A	13.68	2.8795	0.3578	6,651	9,151	−2,500
Do	I	B	16.35	4.0180	.3547	9,491	10,161	−670
Do	I	C	13.63	5.1243	.3561	11,198	10,705	+493
Do	I	D	14.47	6.0858	.3544	12,106	11,405	+701
179. Red clover hay	I	1	14.73	4.4590	.....	8,449	11,420	−2,971
Do	I	2	15.12	3.1439	.....	6,154	10,003	−3,849
Red clover hay and corn mea	I	3	13.52	3.1628	.7347	8,238	10,468	−2,230
Do	I	4	18.36	3.1864	3.4508	17.973	14.413	+3,560
186. Red clover hay	I	1a	20.80	2.9333	.....	5,922	10,597	−4,675
Do	I	1b	24.00	2.9333	.....	5,922	11,321	−5,399
Do	I	2a	16.28	5.0253	.....	10,690	11,268	−578
Do	I	2b	17.60	5.0253	.....	10,690	11,113	−423
Do	I	3a	14.87	4.1391	.....	8,614	10,605	−1,990
Do	I	3b	17.38	4.1391	.....	8,614	10,677	−2,062
200. Timothy hay and grain mixture No. 1	A	1	12.58	2.6079	1.7920	10,721	9,324	+1,397
Do	A	2	13.87	2.6309	4.1727	17,290	12,823	+4,467
Timothy hay	A	3	13.71	2.6472	.....	4,939	7,407	−2,468
Do	A	4	12.20	4.4245	.....	7,981	8,204	−223
Timothy hay and grain mixture No. 1	B	1	11.35	2.4331	1.1947	8,502	8,951	−449
Do	B	2	10.86	2.4539	2.1290	10,922	9,743	+1,179
Timothy hay	B	3	13.62	2.4703	.....	4,751	6,893	−2,142
Do	B	4	7.93	3.8051	.....	7,100	7,468	−368
207. Timothy hay and grain mixture No. 1	A	1	12.09	2.9349	1.9962	12,061	10,171	+1,890
Do	A	2	15.44	2.9487	4.7590	20,553	14,035	+6,518
Timothy hay	A	3	11.78	2.9742	.....	6,235	7,780	−1,545
Do	A	4	11.75	4.8920	.....	10,157	9,501	+656
Timothy hay and grain mixture No. 1	B	1	11.50	2.7608	1.3978	9,443	9,527	−84
Do	B	2	10.98	2.7743	2.6769	14,021	11,532	+2,489
Timothy hay	B	3	10.31	2.7983	.....	5,687	7,925	−2,238
Do	B	4	9.44	4.6299	.....	9,446	9,478	−32
209. Alfalfa hay and grain mixture No. 2	F	1	6.4	1.4615	3.0406	11,362	9,096	+2,266
Do	F	2	8.0	.8935	1.8620	7,331	7,476	−145
Do	F	3	7.7	.5361	1.1120	4,438	6,430	−1,992
Alfalfa hay	F	4	8.2	6.1737	.....	11,399	11,081	+318
Do	F	5	7.8	3.5622	.....	6,623	7,763	−1,140
Do	F	6	11.1	2.2264	.....	3,838	6,737	−2,899
210. Corn stover	D	1	12.70	4.3353	.....	8,523	9,457	−934
Do	D	2	9.02	3.5475	.....	6,986	8,263	−1,277
Do	D	3	8.58	2.5628	.....	4,923	7,193	−2,270
211. Mixed hay	D	1	7.12	6.2042	.....	11,348	11,710	−362
Mixed hay and hominy chop	D	2	9.62	1.7473	1.7637	9,435	9,665	−230
Do	D	3	5.85	3.9105	3.9488	21,406	14,123	+7,283
Mixed hay	D	4	8.70	3.4983	.....	6,958	9,302	−2,344
Do	D	5	9.66	1.7864	.....	3,276	8,020	−4,744
Do	G	1	9.52	6.0920	.....	12,054	11,761	+293
Mixed hay and corn meal	G	2	10.00	.7903	1.5419	6,887	8,245	−1,358
Do	G	3	6.72	2.3832	4.6437	18,894	13,444	+5,450
Mixed hay	G	4	8.34	3.1485	.....	6,217	9,029	−2,812
Do	G	5	7.27	1.6078	.....	3,018	6,983	−3,965
212. Alfalfa hay	H	1	10.13	6.6383	.....	13,337	11,134	+2,203
Alfalfa meal	H	2	9.50	6.6707	.....	13,238	10,946	+2,292
Alfalfa hay	H	3	7.05	5.3203	.....	10,650	9,843	+807
Alfalfa meal	H	4	9.14	5.4078	.....	10,661	9,767	+894
Alfalfa hay	H	5	8.26	3.0524	.....	6,510	7,394	−884
Alfalfa meal	H	6	8.98	3.1549	.....	6,587	6,868	−281

TABLE II.—*Data for computation of net-energy values*—Continued

Experiment No. and feeding stuffs	Animal	Period	Time spent standing per day	Dry matter eaten		Metabolizable energy of ration	Heat production corrected to 12 hours standing	Gain of energy by animal
				Roughage	Concentrates			
			Hours	*Kg.*	*Kg.*	*Cals.*	*Cals.*	*Cals.*
216. Alfalfa hay and starch	J	1	8.79	6.2486	2.5724	20,860	15,623	+5,237
Do	J	2	9.26	2.6454	1.1050	8,854	9,600	−746
Do	J	3	7.57	3.7477	1.5511	12,019	10,924	+1,095
Do	J	4	9.51	1.7540	.7260	5,628	8,026	−2,398
Alfalfa hay	J	5	8.97	7.8930	.....	15,100	12,818	+2,282
Do	J	6	7.13	6.1278	.....	11,954	10,954	+1,000
Do	J	7	8.49	3.5016	.....	6,902	8,315	−1,413
217. Alfalfa hay and grain mixture No. 3	J	1	10.15	1.5076	2.9553	12,269	10,954	+1,315
Do	J	2	13.37	3.0884	6.0579	25,398	16,604	+8,794
Do	*J	3	17.56	3.4395	6.7872	28,747	21,002	+7,745
Do	*J	4	13.01	1.7543	3.4613	14,338	14,187	+151
220. Red clover hay	K	1	11.12	5.9523	.....	11,365	12,151	−786
Do	K	2	11.62	3.9415	.....	7,972	10,300	−2,328
Red clover hay and corn meal	K	3	9.25	1.3279	2.6017	11,292	10,838	+454
Do	K	4	10.27	2.2717	4.3630	18,132	13,899	+4,233
Do	K	5	13.81	.9087	1.7475	7,383	10,001	−2,618

* Fattened.

REVISED METHOD OF COMPUTATION OF NET-ENERGY VALUES

A detailed description of the new method of computing net-energy values, supplemented by a number of examples, showing the advantage of this method over those previously used, has already been published by one of the writers (*11*), and to avoid unnecessary repetition the description here will be general.

The distinguishing feature of this method is that it makes possible the computation of a net-energy value of a feed for each of a series of experimental periods, instead of giving only one value representing results of two or more periods, as accomplished by the earlier methods. The computation is carried out according to the following general procedure:

(1) The daily gain of energy by the animal in each experimental period of a series is computed by subtracting the daily heat production [4] from the metabolizable energy of the ration (see Table 2, last column).

(2) The heat increment per kilogram of dry matter of a feed is computed by comparing the dry matter and the heat production of each period with the dry matter and the heat production of the other periods. The difference in heat production divided by the difference in dry matter of feed consumed (kilogram) gives the increment of heat production per kilogram of dry matter. The values thus obtained are averaged.

(3) The total heat increment caused by the feed is computed by multiplying the total dry matter of the feed (kilogram) by the average heat increment per kilogram of dry matter, as obtained in (2).

(4) The net energy required for maintenance is computed by subtracting the total heat increment caused by the ration, in each period,

[4] Corrected to 12 hours standing and 12 hours lying. This is also implied in the subsequent uses of this term.

from the heat production of the corresponding period. The average of the several determinations is considered to represent the maintenance requirement of the animal during the series of experimental periods.

(5) The toal net energy of each ration is computed by adding to the gain, as obtained in (1), the average net energy required for maintenance, as obtained in (4).

(6) The net-energy value per kilogram of dry matter of feed is computed by dividing the total net energy of the feed by the dry matter (kilogram) of the feed.

(7) The percentage utilization of the metabolizable energy is computed by dividing the total net energy of the feed by its total metabolizable energy, and multiplying the result by 100.

INCREMENTS OF HEAT PRODUCTION PER KILOGRAM OF DRY MATTER OF FEED

The feeds used in the experiments under consideration consisted in some cases of coarse fodder alone, and in others of a mixture of roughage and concentrates (Table II). The increments of heat production per kilogram of roughage alone, or of a uniform mixture of hay and grain, are computed directly, as outlined above, by comparing the periods in which different quantities of the same feed, or of the same mixture of feeds, were consumed.

In the experiments following No. 207 the rations consisted of a uniform mixture of feeds, in the different periods. The increments of heat production, therefore, are computed per kilogram of the mixture, these values being directly usable for the computation of the maintenance requirement of the animal.

In the earlier experiments, however, up to and including No. 207, the hay and grain were not fed in the same proportion in the different periods. In these the heat increments per kilogram of the grain have been computed by making use of the hay periods in the comparisons.

The heat increment values of the feeds, as obtained by the use of the heat production corrected to 12 hours standing and 12 hours lying, according to the new method, and the averages of each series, are set forth in Table III.

TABLE III.—*Increments of heat production per kilogram of dry matter of feed*

[Values in parenthesis are not included in averages]

Experiment No.	Animal	Feeding stuff	Periods compared	Heat increment per kilogram of dry matter
				Cals.
174	I	Timothy hay	D and A	704
		Do	C and A	693
		Do	B and A	889
		Do	D and B	602
		Do	C and B	491
		Do	D and C	730
		Average		685
179	I	Red clover hay	1 and 2	1,077
		Corn meal	2 and 4	[a] 1,264
186	I	Red clover hay	1a and 3a	(7)
		Do	2a and 3a	(748)
		Do	1a and 2a	(321)
		Do	1b and 3b	(−534)
		Do	2b and 3b	(492)
		Do	1b and 2b	(−99)
200	A	Timothy hay	3 and 4	(448)
		Grain mixture No. 1	1 and 3	(1,080)
		Do	2 and 1	(1,466)
		Do	2 and 3	(1,300)
	B	Timothy hay	3 and 4	(431)
		Grain mixture No. 1	1 and 3	(1,736)
		Do	2 and 1	(838)
		Do	2 and 3	(1,342)
207	A	Timothy hay	3 and 4	897
	A	Grain mixture No. 1	1 and 3	[a] 1,215
		Do	2 and 1	1,394
		Do	2 and 3	[b] 1,319
		Average		1,309
	B	Timothy hay	3 and 4	848
	B	Grain mixture No. 1	1 and 3	[a] 1,169
		Do	2 and 1	1,559
		Do	2 and 3	[b] 1,355
		Average		1,361
209	F	Alfalfa hay	4 and 5	1,271
		Do	5 and 6	768
		Do	4 and 6	1,100
		Average		1,046
	F	Alfalfa hay and grain mixture No. 2	1 and 2	927
		Do	2 and 3	945
		Do	1 and 3	934
		Average		935
210	D	Corn stover	1 and 2	1,516
		Do	2 and 3	1,087
		Do	1 and 3	1,277
		Average		1,293
211	D	Mixed hay	1 and 4	890
		Do	4 and 5	750
		Do	1 and 5	835
		Average		825
	D	Mixed hay and hominy chop	2 and 3	1,025
	G	Mixed hay	1 and 4	928
		Do	4 and 5	1,328
		Do	1 and 5	1,066
		Average		1,107
	G	Mixed hay and corn meal	2 and 3	1,107

[a] The same value will be obtained by comparing periods 1 and 4.
[b] The same value will be obtained by comparing periods 2 and 4.

TABLE III.—*Increments of heat production per kilogram of dry matter of feed*—Continued

Experiment No.	Animal	Feeding stuff	Periods compared	Heat increment per kilogram of dry matter
				Cals.
212	H	Alfalfa hay	1 and 3	980
		Do	3 and 5	1,080
		Do	1 and 5	1,043
		Average		1,034
		Alfalfa meal	2 and 4	934
		Do	4 and 6	1,287
		Do	2 and 6	1,160
		Average		1,127
216	J	Alfalfa hay	5 and 6	1,056
		Do	6 and 7	1,005
		Do	5 and 7	1,025
		Average		1,029
		Alfalfa hay and starch	1 and 2	1,188
		Do	1 and 3	1,334
		Do	1 and 4	1,198
		Do	3 and 2	855
		Do	2 and 4	1,239
		Do	3 and 4	1,028
		Average		1,140
217	J	Alfalfa hay and grain mixture No. 3	1 and 2	1,206
	*J	Do	3 and 4	1,360
220	K	Red clover hay	1 and 2	921
	K	Red clover hay and corn meal	3 and 4	1,132
		Do	4 and 5	980
		Do	3 and 5	(658)
		Average		1,056

* Fattened.

VARIABILITY OF HEAT-INCREMENT VALUES

The variations in the heat increments per kilogram of dry matter exhibited in Table III call for a consideration of their true significance and of several factors possibly contributing to apparent discrepancies.

These variations are not to be considered in the same light as variations in other determinations, for example, of digestibility or of metabolizable energy. We may have, for instance, in two periods, independent determinations of metabolizable energy per kilogram of feed, but, in connection with the same, only one heat-increment value. On the other hand, three periods on the same feed make possible three comparisons, and, therefore, as many determinations of heat increment; and four periods make possible six comparisons, and, therefore, six determinations of heat increment, while no one of these heat-increment values represents the result of a single period. Variations in heat increments, therefore, can not be referred to the heat production of individual periods. The extent of these variations, and their effects on the average heat increment per kilogram of feed, depend not only upon the magnitude of the errors in the values rep-

resenting heat production, but also upon the periods in which the errors occurred, and upon the differences in feed between the periods compared. This may be illustrated by the following hypothetical example involving three experimental periods:

Let H_1, H_2, and H_3 represent the heat production of Periods I, II, and III, respectively. The ration of Period III is the smallest. The difference in dry matter consumed between Periods II and III is 1 kg.; between I and II, 3 kg.; and between I and III, 4 kg. The heat increments per kilogram of dry matter would therefore be as follows:

Periods compared	Heat increment per kilogram dry matter
I and II	$\frac{H_1-H_2}{3}$
II and III	$\frac{H_2-H_3}{1}$
I and III	$\frac{H_1-H_3}{4}$

Let three cases be considered:

(1) If H_1 alone is in error by ± 300 Cals.
(2) If H_2 alone is in error by ± 300 Cals.
(3) If H_3 alone is in error by ± 300 Cals.

If h represents the true heat increment per kilogram of dry matter of the feed, we will have the following heat-increment values in the three cases just mentioned:

Periods compared	Difference in feed (dry matter)	Heat increments per kilogram of dry matter		
		Case 1	Case 2	Case 3
	Kg.	*Cals.*	*Cals.*	*Cals.*
I and II	3	$h \pm 100$	$h \mp 100$	h
II and III	1	h	$h \pm 300$	$h \mp 300$
I and III	4	$h \pm 75$	h	$h \mp 75$
Average		$h \pm 58$	$h \pm 67$	$h \mp 125$

The difference between the extreme heat-increment values in case 1 is 100 Cals., while it is 400 Cals. in case 2, and 300 Cals. in case 3, although the magnitude of the assumed error in the heat production is the same in each case. The effect of this assumed error on the average heat-increment value is also different in the three cases.

Especial attention is called to the relative magnitude of the deviations from the true value (h) in the different comparisons of the periods in which the error is involved. The deviation is greatest where the difference in feed is smallest. On this account it seems very desirable that the feed of the different periods to be compared shall differ in quantity as widely as practicable; and in some cases in which the evidence is clear as to the location of the errors it seems permissible to exclude from the average the heat-increment values based upon small differences in feed.

Accordingly, we have excluded Period III of experiment 179 from the computation of the average heat-increment value of corn meal, because of the small quantity of meal represented in this period, and we have also excluded from the average the heat-increment value of clover hay and corn meal as obtained by comparing Periods III and V in experiment 220, because of the small difference in feed.

Among the sources of error in the heat production, and consequently in the heat increments, in some of the experiments here considered, two require especial mention. These are (1) the refusal of feed in some of the periods of heavy consumption, and (2) the probability that the temperature of the calorimeter was below the critical temperature for the animal in some of the periods in which the subjects on submaintenance rations suffered extensive losses of energy from the body.

The amounts of feed refused have been reported in the publication of the details of these experiments (*1*, *2*, *3*, *4*, *6*, *7*, *8*, *9*) and will not be repeated here. In experiments 209 and 210, which have been published only in condensed form (*6*) and not in full detail, there were considerable amounts of feed refused during some of the periods. During Period IV, experiment 209, the animal was offered daily 6.8037 kg. of dry matter of alfalfa hay, but the average daily amount eaten was only 6.1737 kg. (See Table II.) During Period I of the same experiment the animal rejected 147.6 grams of dry matter of hay and 9.3 grams of grain. In experiment 210 the animal was offered daily during Period I 7.6303 kg. of dry matter of corn stover, and during Period II 4.2040 kg., but the average daily amounts eaten during these periods were 4.3353 kg. and 3.5475 kg., respectively.

In comparing the heat production and the rations of the different periods, for the derivation of the heat increments, the amounts of feed refused were in each case subtracted from the total offered. It is, however, readily understandable that such a procedure may not always be effective in accomplishing the desired correction for refusal of feed, when it is considered that the balance of matter represents an average of 10 days' feeding, while the heat production is derived from only two days. It is clear that the success or failure of such an attempt at correction of the heat production must depend to a large extent on whether the refusal of feed was uniform throughout the entire digestion period, including the calorimeter days.

In connection with the effect of refusal of feed on the heat production, its effect on the determination of metabolizable energy, which also is involved in the computation of net-energy values, must also be mentioned.

A refusal of feed occurring early enough in the collection period to be wholly reflected in the fecal outgo of this period would be corrected for by subtraction of the amount of the refused feed from the amount of the feed offered, but if the decrease in fecal outgo consequent upon the refusal of feed does not all occur prior to the termination of the collection period, the attempt at correction of the balance of matter by subtraction of the amount of feed refused from the amount of feed offered would at best be incomplete, and, under some conditions, might actually introduce into the balance a new error of the extent of the entire amount of the refused feed thus subtracted from the amount of feed offered. In the experimental practice of this institute the

result has been, in all probability, rather commonly at least, intermediate between these extremes, there being no way to determine positively the net effect of this effort to correct for refusal of feed. In fact, refusal of feed introduces most perplexing complications, and, if considerable in amount, is fatal to the significance of the results.

For present purposes the writers leave this matter as it stands, with a citation of the published figures representing amounts of feed refused, and an acknowledgment of the fact that this has been a source of error—commonly a minor one, it is true, but sometimes extensive.

As for the bearing of the critical temperature in this connection, there is evidence to suggest, in some periods of restricted feed intake, that the heat incident to the vital processes and the utilization of the feed was insufficient to maintain the normal temperature of the animal, the subject being obliged, therefore, to oxidize body substance to make good the deficit. In such a case the heat increment would exceed the amount derived from the feed, and so would be in error as related to the feed alone.

In the present lack of definite evidence as to what the critical temperature for cattle is, under the various conditions in accord with which it is believed to vary, we are unable to say positively that this factor is responsible for certain anomalous results obtained, but the presumptive evidence is of such weight, in the writers' judgment, as to warrant withholding some of these results until further information as to critical temperatures shall remove the question as to their significance.

In other experiments, however, in which there was ground for suspecting error on this account, it was possible to use the results by computing the maintenance requirements from the results of other experiments with the same subjects, as will be explained in the following paragraph.

MAINTENANCE REQUIREMENT OF NET ENERGY

In computing the net energy required for maintenance, the general procedure as outlined was followed, except in two series of experiments, namely Nos. 186 and 200. In these cases, on account of the ground for suspicion that the temperature in the calorimeter was below the critical for the subjects in some of the periods, we have computed the maintenance requirements from data obtained in other experiments with the same animals. In so doing the writers computed this quota in proportion to the two-thirds power of the live weight. Thus the maintenance requirement of steer I in experiment 186 was computed from the maintenance of this animal as determined in experiment 179, while the maintenance requirements of steers A and B in experiment 200 were computed from the maintenance requirements of these animals as determined in experiment 207.

In this connection it should be stated that the reason for not including in this paper the experiments with the young growing steers, namely experiments Nos. 190 and 208, to which reference has already been made, is that these experiments are also subject to the same suspicion of having been affected by subcritical temperatures, and, as the writers have no adequate basis for computing the net-energy

requirement for maintenance of these young animals, the net-energy estimations derivable from the results are withheld for the time being.

Table 4 gives the maintenance requirements of net energy, as determined in the different periods, and the averages for the series. It should be noted that in averaging the maintenance requirements for the series of experimental periods only one value is given for two periods in those cases in which only two periods were the basis for determining the heat-increment value of the feed. This is done because in such cases only one comparison is possible, and only one heat-increment value, therefore, is obtained. The value for maintenance must be considered to be the same in the two periods.

TABLE IV.—*Maintenance requirements of net energy*

Experiment No.	Animal	Net energy required for maintenance							
		In successive periods							Average
		Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
174	I	6,794	7,027	6,812	6,855				6,872
179	I	6,617			6,619				6,618
186	I								[a] 6,981
200	A								[b] 4,365
200	B								[b] 4,692
207	A	4,925	5,160	5,113					5,066
207	B	5,284	5,536	5,552					5,457
209	F	4,887	4,900	4,889	4,623	4,037	4,408		4,624
210	D	3,851	3,676	3,879					3,802
211	D	6,592	6,067		6,416	6,546			6,405
211	G	5,017	5,664		5,544	5,203			5,357
212	H	4,270	3,428	4,342	3,672	4,238	3,312		3,877
216	J	5,567	5,325	4,883	5,199	4,696	4,648	4,712	5,004
217	J	5,573							5,573
217	[c] J			7,094					7,094
220	K	6,669		6,688	6,893	7,196			6,861

[a] Computed from experiment 179. [b] Computed from experiment 207. [c] Fattened.

VARIATIONS IN THE VALUES FOR MAINTENANCE

An examination of the values for maintenance requirement of net energy in each of the series of experiments from which the averages are derived, shows that the differences are apparently due (Table IV) not to differences in kind of ration used (hay, or hay and grain), in the different periods of the same series, or to variations in live weight of the animal, but to accumulated errors of work. Thus, in experiment 211 the maintenance requirement of net energy for steer D seems to be less in Periods II and III, on hay and grain rations, than in the other periods in which hay alone was fed, while the maintenance requirement of steer G appears to be greater in the hay and grain periods than in the hay periods. In experiments 209, 216, and 220, the maintenance requirements computed from the hay and grain rations are, in general, greater than those computed from the hay rations, but in some of these the differences are only slight. In experiment 212 the maintenance requirements computed from the alfalfa meal rations (Periods II, IV, VI) are considerably less than those computed from the alfalfa hay rations (Periods I, III, V). These differences can hardly be due to difference in kind of feed. As to the possible effect of variations in the live weight of the animals, in the different periods, on the results of the computation

of the maintenance requirement of net energy, the writers would suggest that, with the exception of experiment 217, the experimental periods were short (three or four weeks only), while the changes in the amount and kind of feed consumed were considerable. It seems probable, therefore, that the larger part of the variation in weight must be ascribed to differences in the content of the alimentary tract, and that the actual effect on the basal metabolism was but slight. In view of all these considerations, the writers are inclined to ascribe the apparent variations in the maintenance requirement of net energy, in the different experimental periods of a series, in Table IV, to the cumulative effect of experimental errors, and to consider the average of the several determinations as applying to each of the individual experimental periods.

In experiment 217 the animal was actually fattened during the transition period of more than three months, between Periods II and III, and made considerable gain in weight (see Table I). In this experiment the maintenance requirements of the animal in the unfattened condition and the fattened condition have been computed separately, the values being as shown for Periods II–III and III–IV, respectively.

NET-ENERGY VALUES OF FEEDS

The total net energy of the rations is obtained, by addition, of the average of the determinations of net-energy requirement for maintenance, as reported in Table IV, and the gains of energy, as given in Table II. These total net-energy values, divided by the dry matter of the rations, give the net-energy values per kilogram of dry matter of the rations. The net-energy values of the grains are computed by subtracting from the total net energy of the mixed ration the net-energy equivalent of the hay, by the use of a value previously determined in the hay rations. The remainder represents the net energy of the grain, and by dividing this by the kilograms of dry matter of grain the net-energy value per kilogram of grain is obtained.

We have also computed, in each case, the percentage utilization of the metabolizable energy. This is another way of expressing the net-energy value of a feed, and is obtained by dividing the net energy of the feed by its metabolizable energy (instead of the dry matter), and multiplying the result by 100. The net-energy values, on both bases, are presented in Table V.

TABLE V.—*Net-energy values of feeding stuffs*

[Values in parentheses are not included in averages]

Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
		Cals.	*Per cent*
Timothy hay:			
174	I-A	1,275	65.98
	I-B	1,371	65.45
	I-C	1,301	65.90
	I-D	1,130	62.36
200	A-III	(717)	(38.41)
	A-IV	(936)	(51.90)
	B-III	(1,032)	(53.67)
	B-IV	(1,136)	(60.90)
207	A-III	1,184	56.47
	A-IV	1,170	56.34
	B-III	1,150	56.60
	B-IV	1,172	57.43
Average, omitting experiment 200		1,219	60.82
Alfalfa hay:			
209	F-IV	800	43.35
	F-V	978	52.60
	F-VI	775	44.95
212	H-I	916	45.59
	H-III	881	43.99
	H-V	981	45.99
Alfalfa meal:			
212	H-II	925	46.61
	H-IV	882	44.76
	H-VI	(1,140)	(54.61)
Alfalfa hay:			
216	J-V	923	48.25
	J-VI	980	50.23
	J-VII	1,026	52.03
Average, including alfalfa meal		915	47.12
Corn meal:			
179	I-III	(2,314)	(79.22)
	I-IV	2,165	63.11
211	G-II	2,149	61.93
	G-III	1,882	61.29
220	K-III	2,257	67.65
	K-IV	1,977	63.13
	K-V	1,863	58.18
Average		2,049	62.55
Red clover hay:			
179	I-I	818	43.16
	I-II	881	45.00
186	I-Ia	(786)	(38.94)
	I-Ib	(540)	(26.73)
	I-IIa	(1,274)	(59.90)
	I-IIb	(1,305)	(61.35)
	I-IIIa	(1,206)	(57.93)
	I-IIIb	(1,188)	(57.10)
220	K-I	1,021	53.45
	K-II	1,150	56.86
Average, omitting experiment 186		908	49.62
Mixed hay:			
211	D-I	974	53.25
	D-IV	1,160	58.34
	D-V	930	50.70
	G-I	927	46.86
	G-IV	808	40.94
	G-V	866	46.12
Average		944	49.37

Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
		Cals.	*Per cent*
Corn stover:			
210	D-I	662	33.65
	D-II	712	36.14
	D-III	598	31.14
Average		657	33.64
Grain mixture No. 3:			
217	J-I	1,833	58.02
	J-II	1,874	58.55
Average		1,854	58.29
Grain mixture No. 1:			
200	A-I	(1,853)	(55.95)
	A-II	(1,526)	(51.11)
200	B-I	(1,344)	(41.27)
	B-II	(1,508)	(51.20)
207	A-I	1,754	59.00
	A-II	1,705	56.34
207	B-I	1,551	56.72
	B-II	1,765	56.43
Average, omitting experiment 200		1,694	57.12
Grain mixture No. 2:			
209	F-I	1,857	64.77
	F-II	1,997	65.09
	F-III	1,957	62.75
Average		1,937	64.20
Red clover hay and corn meal:			
220	K-III	1,862	64.78
	K-IV	1,672	61.18
	K-V	1,597	57.47
Average		1,710	61.14
Hominy chop:			
211	D-II	2,490	71.48
	D-III	2,455	69.06
Average		2,473	70.27
Starch:			
216	J-I	1,610	47.58
	J-II	1,517	45.19
	J-III	1,574	51.61
	J-IV	(1,231)	(40.34)
Average		1,567	48.13
Mixed hay and corn meal:			
211	G-II	1,715	58.07
	G-III	1,538	57.20
Average		1,627	57.64
Alfalfa hay and grain mixture No. 3:			
217	J-I	1,543	56.14
	J-II	1,571	56.57
	[a]J-III	1,451	51.62
	[a]J-IV	1,389	50.53
Average		1,489	53.72

[a] Fattened.

TABLE V.—*Net-energy values of feeding stuffs*—Continued

Feeding stuff and experiment No.	Animal and period	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
		Cals.	*Per cent*
Red clover hay and corn meal:			
220	K–II	1,862	64.78
	K–IV	1,672	61.18
	K–V	1,597	57.47
Average		1,710	61.14
Mixed hay and hominy chop:			
211	D–II	1,759	65.45
	D–III	1,742	63.94
Average		1,751	64.70
Alfalfa hay and grain mixture No. 2:			
209	F–I	1,530	60.64
	F–II	1,625	61.10
	F–III	1,597	59.31
Average		1,584	60.35
Alfalfa hay and grain mixture No. 3:			
217	J–I	1,543	56.14
	J–II	1,571	56.57
	[a]J–III	1,451	51.62
	[a]J–IV	1,389	50.53
Average		1,489	53.72
Alfalfa hay and starch:			
216	J–I	1,161	49.09
	J–II	1,135	48.09
	J–III	1,151	50.74
	J–IV	1,051	46.30
Average		1,125	48.56

AVERAGE NET-ENERGY VALUES

It will be noted in Table V that some of the values are in parenthesis. These values are excluded from the averages. Among these are all of the net-energy values obtained for clover hay in experiment 186, and for timothy hay in experiment 200, although some of the results fall within the range of variation of the values obtained in other experiments with the same feeds. These have been excluded for the reason that they rest upon a somewhat less substantial basis than the other determinations, the maintenance requirement having been computed from results of other experiments with the same animals.

The results on corn meal in Period III of experiment 179 (*2*), and the results on starch in Period IV of experiment 216 (*9*) are given in parentheses because of the small quantities of these feeds represented.

The values for alfalfa meal in Period VI of experiment 212 have been excluded from the average because of the extent of their divergence from the other values of the same series.

The net-energy values of grain mixture No. 3 in experiment 217 have been computed from the mixed feed of hay and grain in Periods I and II, representing the unfattened condition of the animal, by the use of the net-energy value of the hay obtained in experiment 216 with the same animal.

For convenience of reference, the average net-energy values of Table V have been assembled in Table VI.

TABLE VI.—*Average net-energy values of feeding stuffs*

Feeding stuffs	Net energy per kilogram of dry matter	Percentage utilization of metabolizable energy
	Cals.	*Per cent*
Timothy hay	1,219	60.82
Red clover hay	968	49.62
Mixed hay	944	49.37
Alfalfa hay [a]	915	47.12
Corn stover	657	33.64
Corn meal	2,049	62.55
Grain mixture No. 1 [b]	1,694	57.12
Grain mixture No. 2 [c]	1,937	64.20
Grain mixture No. 3 [d]	1,854	58.29
Hominy chop	2,473	70.27
Starch	1,567	48.13
Mixed hay and corn meal, 1:2	1,627	57.64
Red clover hay and corn meal, 1:2	1,710	61.14
Mixed hay and hominy chop, 1:1	1,751	64.70
Alfalfa hay and grain mixture No. 2, 1:2	1,584	60.35
Alfalfa hay and grain mixture No. 3, 1:2	1,489	53.72
Alfalfa hay and starch, 2:4:1	1,125	48.56

[a] Includes alfalfa meal.
[b] 1 part wheat bran, 3 parts corn meal, 3 parts old process linseed oil meal.
[c] 6 parts corn meal, 3 parts crushed oats, 1 part coarsely ground linseed cake.
[d] 6 parts corn meal, 2 parts wheat bran, 1 part cottonseed meal.

SUMMARY

The latest evidence in relation to the determination of the net energy of feeds, in accord with Armsby's conception, having led (1) to a new and greatly improved method of correcting the heat production of the experimental animal to a standard day, in relation to the position of the subject as to standing and lying, and (2) to an important change in the method of computation of the net-energy value, it has become necessary to recompute and to correct the net-energy values of feeds, for the maintenance and body increase of steers, which have been published from the Institute of Animal Nutrition of the Pennsylvania State College (*1, 2, 3, 4, 6, 7, 8, 9*). The corrected net-energy values are as assembled in Table VI.

LITERATURE CITED

(1) ARMSBY, H. P., and FRIES, J. A.
1903. THE AVAILABLE ENERGY OF TIMOTHY HAY. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 51, 77 p., illus.

(2) ——— and FRIES, J. A.
1905. ENERGY VALUES OF RED CLOVER HAY AND MAIZE MEAL. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 74, 64 p., illus.

(3) ——— and FRIES, J. A.
1908. THE AVAILABLE ENERGY OF RED CLOVER HAY. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 101, 61 p., illus.

(4) ——— and FRIES, J. A.
1911. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr., Bur. Anim. Indus. Bul. 128, 245 p., illus.

(5) ——— and FRIES, J. A.
1913. THE INFLUENCE OF STANDING OR LYING UPON THE METABOLISM OF CATTLE. Amer. Jour. Physiol. 31: 245–253.

(6) ——— and FRIES, J. A.
1915. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. Jour. Agr. Research 3: 435–491 illus.

(7) ARMSBY, H. P., FRIES, J. A., and BRAMAN, W. W.
1916. ENERGY VALUES OF RED-CLOVER HAY AND MAIZE MEAL. Jour. Agr. Research 7: 379–387.
(8) ——— and FRIES, J. A.
1917. ENERGY VALUES OF HOMINY FEED AND MAIZE MEAL FOR CATTLE. Jour. Agr. Research 10: 599–613.
(9) ——— and FRIES, J. A.
1918. NET ENERGY VALUES OF ALFALFA HAY AND OF STARCH. Jour. Agr. Research 15: 269–286.
(10) FRIES, J. A., and KRISS, M.
1924. METABOLISM OF CATTLE DURING STANDING AND LYING. Amer. Jour. Physiol. 71: 60–83.
(11) KRISS, M.
1925. AN IMPROVED METHOD OF COMPUTATION OF NET-ENERGY VALUES OF FEEDING STUFFS. Jour. Agr. Research 31: 469–484.

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